CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The Niger Delta ecosystem of Nigeria is subjected to man-induced changes and seriously threatened by increasing environmental deterioration. The aquatic ecosystem of the region faces increasing ecological and toxicological problems from the release of petroleum pollutants (John and Okpokwasili, 2012). Besides this direct pollution, the occasionally pipeline leaks, transportation accidents, storage tank ruptures and refining petroleum is further intensifying the pollution of this area. Most of these compounds are considered as carcinogenic, mutagenic and potent immunotoxicants and classified as priority environmental pollutant by the US Environmental Protection Agency (Rajaei *et al*., 2013).

Environmental contaminants such as hydrocarbons, heavy metals and pesticides have been known to have direct toxic effects when released into the aquatic environment. There is a direct link between surface water and sediment contamination. Accumulated heavy metals or organic pollutants in sediment could be released back into the water with deleterious effects on human health (Taiwo *et al.*, 2012). These heavy metals have a marked effect on the aquatic flora and fauna. They enter through bio-magnification into the food chain and humans are eventually affected as well. Contamination by heavy metal pollution is the long lasting escalating predicament of oceans, lakes and rivers. The accumulation of heavy metals such as Cadmium (Cd), Lead (Pb), Nickel (Ni), Chromium (Cr), Vanadium (V) and Zinc (Zn) in fish, oysters, sediments and other aquatic living organisms in the ecosystems have been reported globally (Adenike, 2014). These pollutants and untreated industrial effluents can pose a major risk to the environment and aquatic life. For these reasons, many assays especially chemical and biological, have been developed to meet the demand of screening for toxic substances. Unlike some other tests, Marine Algaltoxkit, Phytotoxicity test, Artox kit M and Toxi-

ChromoTest™biological assays detect active toxins and do not usually require a lengthy process of sample preparation (EBPI, 2016).

Xylenes are monocyclic aromatic compounds with two methyl groups attached to the benzene ring. Xylenes exist in three isomeric forms ortho - or o -xylene, meta - or m-xylene and para-xylene or p-xylene (Alberta Environment, 2004). Studies have shown that xylene is well-absorbed by inhalational, oral and to some extent by the dermal route (Rajan and Malathi, 2014). Early studies found that exercise increased the amount of xylene absorbed. Once absorbed, xylene enters into the blood and gets distributed throughout the body. The biotransformation of xylene regardless of the isomer/route of administration proceeds through the oxidation of a side chain methyl group by mixed function oxidases in the liver to form methyl benzoic acids that conjugate with glycine to yield methyl hippuric acid, which is excreted in urine. Most of the xylene that enters the body leaves within 18 hours after the end of the exposure. Following prolonged exposure especially by occupational means it is likely to get accumulated chiefly in the muscle and adipose tissues. Acute (short-term) inhalation exposure to mixed xylenes in humans' results in irritation of the eyes, nose, and throat, gastrointestinal effects, eye irritation, and neurological effects. Chronic (long term) inhalation results primarily to central nervous system (CNS) effects, such as headache, dizziness, fatigue, tremors, and incoordination; respiratory, cardiovascular, and kidney effects have also been reported. EPA has classified mixed xylenes as Group D, not classifiable as human carcinogen (Jacobson and Mclean, 2003; Kandyala *et al.*,2010; Rajan and Malathi, 2014). Several species of bacterial genera *Pseudomonas, Serratia, Marinobacter, Providencia, Alcaligenes, Bacillus* and *Sphingomonas* have been found highly capable of degrading xylene (Amer *et al.*, 2015; Fagbemi and Sanusi, 2017).

Polycyclic Aromatic Hydrocarbons (PAHs) are found in considerable amounts in crude oil and oily effluents of petroleum refineries. Low molecular weight (LMW) PAHs are relatively volatile, high soluble in water and more degradable than high molecular weight (HMW) PAHs (Babita *et al.*, 2013). Anthracene, together with other polycyclic aromatic hydrocarbons (PAHs), is a persistent and toxic soil contaminant. Anthracene is sparingly soluble in water, highly resistant to nucleophilic attack and hence, recalcitrant to biodegradation and accumulate easily in the ecosystem. Anthracene a tricyclic aromatic hydrocarbon causes many problems associated with health and environmental impact. In powdered form, it causes irritation to the eyes, noses or lungs and is a probable inducer of tumors. Once anthracene enters the body, it appears to target the skin, stomach, intestines and the lymphatic system. It may even cause burning, itching and edema. It is released due to incomplete combustion of fuels present in automobiles. It is found in high concentrations in the PAH contaminated sediments, surface soils and waste sites. This hydrophobic contaminant is widely distributed in the environment, occurring as natural constituent of the fossil fuels and their anthropogenic pyrolysis products. Anthracene exhibits toxicity to fish, algae and shows bioaccumulation in the food chain. Anthracene serves as a signature compound to detect PAH contamination, since its chemical structure is found in carcinogenic PAHs. It has also been used as a model PAHs to determine the factors that affect the bioavailability, biodegradation potential and the rate of microbial degradation of the PAHs in the environment. Due to its low solubility, most ofthe researchers attempt to remove anthracene in soil/sediment. Several species of bacterial genera *Pseudomonas, Salmonella, Nocardia, Mycobacterium, Cunninghamella, Rhodococcus, Beijerinckia, Lysinibacillus, Bacillus* and *Sphingomonas* have been found highly capable of degrading anthracene (Lily *et al.*, 2013; Swaathy *et al.*,2014a, Akinbankole *et al.*, 2015).

Pyrene is a high molecular weight four benzene-rings polycyclic aromatic hydrocarbon (PAH) that has low biodegradability and high persistence in the environment and has been listed as a priority pollutant by the United States Environmental Protection Agency. Pyrene ($C_{16}H_{10}$) was mainly detected in environmental samples and crude oil resulting from incomplete combustion and used as an indicator for PAH-contaminated waste monitoring. Due to its chemical structure is highly recalcitrant and resistant to microbial degradation. Pyrene can easily enter the human body through skin contact and inhalation. It is toxic to body organs, including the kidney and liver. Pyrene could become a neurotoxicant and skin or sense organ toxicant, very toxic to terrestrial and aquatic lives with long lasting effects. Pyrene is not a genotoxic compound by itself but it has four aromatic cores that are found in several PAH carcinogens, including benzo pyrene, indeno (*1, 2, 3 - cd*) pyrene and 1-nitropyrene. Due to its toxic characteristic and xenobiotic property, its removal from contaminated soils and environmental samples had caught attention. Various researches had been carried out for pyrene removal through bioremediation techniques, including application of fungi and bacteria (Ceyhan, 2012; Kafilzadeh *et al*., 2012; Teh and Hadibarata, 2014). Several species of bacterial genera *Pseudomonas, Alcaligenes, Mycobacterium, Rhodococcus, Corynebacterium, Diaphorobacter,Pseudoxanthomonas, Bacillus vallismortis et cetra* have been found highly capable of degrading pyrene (Babita *et al.*, 2013).

Physical and chemical methods like volatilization, photooxidation, chemical oxidation, and bioaccumulation are rarely successful in rapid removal and in cleaning up PAHs, and also these methods are not safe and cost effective when compared to microbial bioremediation (Dasgupta *et al*., 2013). A better way is to use biodegradation. Thus, much research has focused on the biological degradation of aromatics hydrocarbons (Ahs) through metabolism and co-metabolism. The degradation process involves enzyme machinery: dehalogenases,

dehydrogenases and hydrolases system (Nakamura *et al*., 2014). Bioremediation is a cost-effective and sustainable biotechnology for the treatment of contaminated coastal and marine sites (Paniagua-Michel and Rosales, 2015). Over twenty genera of bacteria of marine origin have been documented to be hydrocarbon degrading. Bacteria belonging to subphyla α -, β -, and δ - proteobacteria are well established to be of such nature (Dasgupta *et al*., 2013).

One of the major factors that impede the process of bioremediation is bioavailability of hydrophobic contaminants to the hydrocarbon utilising microorganisms due to poor solubility, leading to its accumulation with accompanying toxic and carcinogenic effects (Dasgupta *et al*., 2013; Vyas and Dave, 2011). Different microorganisms were shown to overcome the bioavailability limitations, by possessing multiple adaptations to facilitate oil degradation procedures such as the synthesis of biosurfactants or emulsifiers and biofilm formation, processes that enhance the bacterial adhesion to hydrocarbons, increasing their solubility and thus promoting their degradation (Amer *et al.*, 2015).

Plasmid or chromosomal mediated degradation can be useful and effective during the remediation of crude oil - contaminated sites. Many bacterial strains have genetic determinants of resistance/degradative abilities to pollutants. These determinants are often found on plasmids, chromosomes and transposons. The plasmid profiles of strains of several bacterial genera: *Pseudomonas*, *Rhodococcus*, *Micrococcus*, *Citrobacter koseri*, *Serratia*,*Nitrosomona*s, *Nitrobacter*and *Bacillus* isolated from petroleum-contaminated soils and sediments selected for their capacities to grow in the presence of petroleum and some aromatic hydrocarbons have been determined and the biodegradative abilities have proved to be plasmid related (Fagbemi and Sanusi, 2017; Mirdamadian *et al.*, 2010; John and Okpokwasili, 2012).

The challenge facing scientists and industrialists alike today is tackling this problem of environmental degradation in a safe, environmentally sound manner with rational cost implications. A technology that has been intensively studied is bioremediation (Adams *et al*., 2015). There are limited information on the toxicological profiles and microbial degradation of aromatic hydrocarbons including xylene, anthracene and pyrene in crude oil - impacted Niger Delta ecosystem and hence necessitated this study.

1.2Statement of Problems

1. The Niger Delta ecosystem of Nigeria is subjected to man-induced changes and seriously threatened by increasing environmental deterioration (John and Okpokwasili, 2012).

2. The aquatic ecosystem of the region faces increasing ecological and toxicological problems from the release of petroleum pollutants and has almost become an ecological wasteland (John and Okpokwasili, 2012).

3. Aromatic hydrocarbons are environmental pollutants with toxic, teratogenic, carcinogenic, genotoxic, and mutagenic properties and so their presence in the environment are of great concern and have deleterious effects on human health (Wanjohi *et al.*, 2015).

4. Xylene which is a member of BTEX compounds is a component of gasoline and aviation fuels that are carcinogenic and neurotoxic to most organisms (Hemalatha and Veeramanikandan, 2011).

5. PAHs have been listed as priority pollutants by USEPA in which anthracene and pyrene are involved and are monitored continuously in industrial effluents disposed into soil and aquatic ecosystems.

6. Contamination by heavy metal pollution has a long lasting escalating predicament on oceans, lakes and rivers (Adenike, 2014).

7. The search for effective and efficient methods of aromatic hydrocarbons, heavy metals and other organic pollutants removal from contaminated sites has intensified (Wanjohi *et al.*, 2015).

8. The physical, chemical and mechanical technologies to remove aromatic petroleum hydrocarbons from contaminated marine environments in most of the cases are unsustainable and can be expensive (Paniagua-Michel and Rosales, 2015).

9. One of the major factors that impede the process of bioremediation is bioavailability of hydrophobic contaminants to the hydrocarbon utilizing microorganisms due to poor

solubility, leading to its accumulation with accompanying toxic and carcinogenic effects (Dasgupta *et al*., 2013; Vyas and Dave, 2011).

10. Some of the major problems facing environmental microbiologists are the ability to isolate hydrocarbonoclastic bacteria (HCB) that possess metabolic traits or multiple adaptations to facilitate aromatic hydrocarbon degradation procedures, such as the synthesis of biosurfactants or emulsifiers and catabolic genes, processes that enhance the bacterial adhesion to hydrocarbons, increasing their solubility and thus promoting their degradation.

1.3Aim of the Study

This study was designed to evaluate the toxicological and bioremediating potentials of bacteria isolated from contaminated marine environments of Rivers State.

1.4Objectivesof the Study

The objectives of this study are to:

1. determine the physico - chemical properties of the sediment and water samples from Abonema Wharf water front, Nembe water side and Onne light terminal flow sampling sites in Rivers State via total aromatic hydrocarbon content, total heavy metal concentration and general parameters analyses.

2. test the acute toxicity potentials of xylene, anthracene and pyrene hydrocarbons in distilled water and sediment/water samples using a battery of toxicity tests via marine algal toxicity test using *Phaeodactylum tricornutum* (microalga), phototoxicity test using *Sinapsis alba* (mustard seeds), *Artemia* toxicity test using *Artemia franciscana* (brine shrimps) and Toxi - Chromotest using genetically engineered *E. coli*(bacterium).

3. establish the sub - chronic health effects of xylene, anthracene and pyrene hydrocarbons in Wistar Albino mice at different concentrations (20 mg/kg – 2,000 mg/kg) through oral

administration after 35 days via body and organ weight determination, haematological, biochemical and histopathological analyses.

4. carry out bacteriological analyses on the marine sediment and water samples inorder to enrich, isolate and quantify the bacterial degraders of xylene, anthracene and pyrene hydrocarbons viaenrichment culturing technique and total viable count technique.

5. characterize and identify the best bacterial degraders of xylene, anthracene and pyrene hydrocarbons from the marine contaminated sites via screening and selection technique as well as their morphological, biochemical and molecular characteristics.

6. determine the abilities of the best bacterial degraders to possess multiple resistances to different concentrations of xylene, anthracene, pyrene, other petroleum products and heavy metals via concentration effectdetermination and substrate specificity test.

7. examine therelative degradative potentialsof the bacterial degraders using degradation assay.

8. identify the metabolites/products formed/degraded during the degradation processes via Thin Layer Chromatography (TLC) and Gas Chromatography- Mass Spectroscopy (GC–MS) analyses.

9. detect the existence of catabolic and surfactant genes in the isolated aromatic degrading bacterial strains via PCR method with specific primers.

10. determine the location of catabolic and surfactant genes in the isolated aromatic degrading bacterial strains via plasmid curing analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nigerian Coastal Area

Nigeria has a coastal line of approximately 450km towards the Atlantic Ocean lying between latitude 4°15' to 4°50' and longitude 5°25' to 7°37' with a land mass of about 28000 sq/km area within the coastal region. The surface area of the continental shelf is 46300 sq/km. The coastal areas consist of freshwater swamp, mangrove swamp, beach ridges, sand bars, lagoons marshes and tidal channels. Nigeria has a total land mass of 923,768 sq/km; 918,768 sq/km being terrestrial land and 13000 sq /km being aquatic. The coastal area is humid with amean average temperature of 24 - 32 °C and coastal areahas an average annual rainfall ranging between 1,500 - 4,000 m. Nigeria has two large rivers; the Niger-Benue and the Chad River. There are several rivers that channel into the Atlantic Ocean directly, all other flowing waters flow into the Chad basin or into the lower Niger to the sea eventually. The Niger Delta is located in the Atlantic coast of Southern Nigeria and is the world's second largest delta with a coastline of about 450 km which ends at Imo river entrance. The region is about 20,000 sq/km as it is the largest wetland in Africa and among the third largest in the world. 2,370 sq/km of the Niger Delta area consists of rivers, creeks, estuaries and stagnant swamps cover approximately 8600 sq/km, the Delta mangrove swamp spans about 1900 sq/km as the largest mangrove swamp in Africa (Ayuba, 2012).

The Niger Delta is classified as a tropical rainforest with ecosystems comprising of diverse species of flora and fauna both aquatic and terrestrial species. The region can be classified into four ecological zones; coastal inland zone, freshwater zone, lowland rainforest zone, mangrove swamp zone and this region is considered one of the ten most important wetlands and marine ecosystems in the world. As of 1991, the National Census estimated about 25 %

of the entire Nigerian population lives within the Niger Delta region. The Niger Delta region has a steady growing population of approximately 30 million people as of 2005, accounting for more than 23 % of Nigeria's total population (Ayuba, 2012).

2.2 Oil Production in Nigeria

Nigeria has been a member of Organization of Petroleum Exporting Countries (OPEC) since 1971. It has the largest natural gas reserve in Africa, has the second largest oil reserve in Africa and is the African continents primary oil producer. As of the 1980's oil revenue provided 90 % of Nigeria foreign exchange earnings and 85 % of the government revenue, with estimated reserves extending beyond 20 - 30 years. Shell D'Arcy the pioneer oil company in Nigeria, which started commercial production in 1958 with a production rate of 5100 barrels per day and a peak production of 2.44 million barrels per day over the next few years. According to NNPC (1984) through OPEC, production rates dropped to 1.5 million barrels per day from the activities of 10 international companies working 122 fields, containing over 970 oil wells. Nigeria has four oil refineries with an estimated total refining capacity of 445,000 barrels per day. The first and oldest being the Port Harcourt refinery, commissioned in 1965. It had an initial capacity of 35,000 barrels per day, which was later expanded to 60,000 barrels per day of light crude oil. The Port Harcourt refinery has a second refinery with a capacity of 150,000 barrels per day. Some authors cited in their studies that the region has about 606 oil fields with 355 situated onshore; 251 situated offshore with 5, 284 drilled oil wells and 7,000km of oil and gas pipelines (Ayuba, 2012).

2.3 Biodiversity in the Niger Delta

The ecosystem of the area is highly diverse and supportive of numerous species of terrestrial and aquatic fauna and flora as well as human life. The Niger Delta has been declared as a key

zone for the conservation of the Western Coast of Africa on the basis of its extraordinary biodiversity. It is estimated that in Nigeria, there are more than 46,000 plant species of which about 205 are endemic, and approximately 484 plants in 112 families are threatened with extinction as well as many animal and bird species. Another estimate is that 24 out of 274 mammals, 10 out of 831 birds and 2 out of 114 reptiles known to exist in Nigeria are endangered. The larger population of the Niger Delta survives on services provided by the ecosystem, agriculture, industry, fishing, food, drinking water, wood, shelter, medicine, employment and aesthetics. All aspect of oil exploration and exploitation has adverse effects on the ecosystem and the local biodiversity. Oil exploration by seismic oil companies involves clearing of seismic lines anddynamiting for geological excavation which affect the aquatic environment. It causes mortality in fauna, turbidity in the water that blockage of gills of the filter feeders in the benthic fauna, reduction of photosynthetic activity caused by the water turbidity that reduces the amount of sunlight penetration. Most incidences of the reported oil spillages have occurred in the mangrove swamp forest, which is one of the most reproductive ecosystems rich in fauna and flora (Ayuba, 2012).

2.4 Oil Spillages

An estimated 9 million - 13 million (1.5 million tons) of oil has been spilled into the Niger Delta ecosystem over the past 50 years; 50 times the estimated volume spilled in Exxon Valdez oil spill in Alaska 1989. The first oil spill in Nigeria was at Araromi in the present Ondo State in 1908. In July 1979, the Forcados tank 6 Terminal in Delta State incidence spilled 570,000 barrels of oil into the Forcados estuary polluting the aquatic environment and surrounding swamp forest. The Funiwa No. 5 Well in Funiwa Field blew out an estimated 421,000 barrels of oil into the ocean from January 17th to January 30th 1980 when the oil flow ceased 836 acres of mangrove forest within six miles off the shore was destroyed. The

Oyakama oil spillage of 10th May, 1980 with a spill of approximately 30,000 bbl. In August 1983, Oshika village in Rivers State witnessed a spill of 5,000 barrels of oil from Ebocha-Brass (Ogada-Brass 24) pipeline which flooded the lake and swamp forest, the area had previously experienced an oil spill of smaller quantity; 500 barrels in September 1979 with mortality in crabs, fish and shrimp. Eight months after the occurrence of the spill, there was high mortality in embryonic shrimp and reduced reproduction due to oil in the lake sediments. The Ogada-Brass pipeline oil spillage near Etiama Nembe in February 1995, spilled approximately 24,000 barrels of oil which spread over freshwater swamp forest and into the brackish water mangrove swamp. The Shell Petroleum Development Company (SPDC) since 1989, recorded an average of 221 spills per year in its operational area involving 7,350 barrels annually. From 1976-1996, a total of 4,647 oil spill incidences spilling approximately 2,369,470 barrels of oil into the environment of which 1,820,410.5 (77 %) were not recovered. Most of these oil spill incidences in the Niger Delta occur on land, swamp and the offshore environment. NNPC estimates 2,300 cubic meters of oil has spilled in 300 separate incidences annually between 1976 -1996 (Ayuba, 2012). Table 2.1 below shows some of the severely oil polluted sites in the Niger Delta region while Table 2.2 shows the number of spills in the Niger Delta between 1979 and 2013.

Table 2.1: Some severely oil - polluted sites in the Niger Delta

Location	Environment	Impacted Area (Ha)	Nature of incidence
Bayelsa State	Highlight		
Biseni	Freshwater Swamp Forest	20	Oil spillage
Etiama Nembe	Freshwater Swamp Forest	20	Oil spillage and fire outbreak
Etelebu	Freshwater Swamp Forest	30	Oil Spill Incidence
Peremabiri	Freshwater Swamp Forest	30	Oil Spill Incidence
Adebawa	Freshwater Swamp Forest	10	Oil Spill Incidence
Diebu	Freshwater Swamp Forest	20	Oil Spill Incidence
Tebidaba	Freshwater Swamp Forest Mangrove	30	Oil Spill Incidence
Nembe creeks	Mangrove forest	10	Oil Spill Incidence
Azuzuama	Mangrove	50	Oil Spill Incidence
9 sites			
Delta State	Highlight		
Opuekebe	Barrier forest island	50	Salt water intrusion
Jones Creek	Mangrove forest	35	Spillage and burning
Ugbeji	Mangrove	2	Refinery waste
Ughelli	Freshwater Swamp Fores	10	Oil Spillage-Well head leak
Jesse	Freshwater Swamp Forest	8	Product leak/burning
Ajato	Mangrove		Oil Spillage Incidence
Ajala	Freshwater Swamp Forest		Oil Spillage Incidence
Uzere	Freshwater Swamp Forest		Oil Spillage Incidence
Afiesere	Freshwater Swamp Forest		Oil Spillage Incidence
Kwale	Freshwater Swamp Forest		Oil Spillage Incidence
Olomoro	Freshwater Swamp Forest		QC
Ughelli	Freshwater Swamp Forest		Oil Spillage Incidence

Ekakpare	Freshwater Swamp Forest		Oil Spillage Incidence
Ughuvwughe	Freshwater Swamp Forest		Oil Spillage Incidence
Ekerejegbe	Freshwater Swamp Forest		Oil Spillage Incidence
Ekerejegbe	Freshwater Swamp Forest		Oil Spillage Incidence
Odimodi	Mangrove Forest		Oil Spillage Incidence
Ogulagha	Mangrove Forest		Oil Spillage Incidence
Otorogu	Mangrove Forest		Oil Spillage Incidence
Macraba	Mangrove Forest		Oil Spillage Incidence
20 sites			
Rivers State	Highlight		
Rumuokwurusi	Freshwater Swamp	20	Oil Spillage
Rukpoku	Freshwater Swamp	10	Oil Spillage
Nisisioken Ogale	Groundwater contamination	41	Oil Spillage/Refined product
Bodo creeks	Swamp, surface and groundwater contamination	3	Oil Spillage/Sabotage/Theft
Ogoniland	Soil and groundwater contamination	>200	Oil Spillage/Sabotage/Theft/ Refined product
5 sites			

Source: Ayuba (2012); Fentiman and Zabbey (2015); Okhumode (2017); Shell Nigeria (2018); UNEP (2011)

Table 2.2: Number of spills in the Niger Delta between 1979 and 2013

Episode	Year	State	Quantity Spilt in Barrels
Forcados Terminal oil spill	1979	Delta	570,000
Funiwa no. 5 Well blow out	1980	Rivers	400,000
Oyakama oil spillage	1980	Rivers	10,000
System 2C Warri, Kaduna pipeline rupture at Abudu	1982	Edo	18,000
Sohika oil spill	1983	Rivers	10,000
Idoho oil spill	1983	Akwa Ibom	40,000
Jones Creek oil spill	1998	Delta	21,000
Jesse oil spill	1998	Delta	10,000
Etiama oil spill	2000	Bayelsa	11,000
Ughelli oil spill	2005	Delta	10,000
Bodo Creek oil spill	2011	Rivers	233,000
Nembe Creek 2 oil spill	2011	Bayelsa	78,100
6"Obigbo North Pipeline at Ogale	2012	Rivers	120,000
36" Nkpoku - Bomu Trunkline at Agbonchia	2012	Rivers	156,800
24" Bomu - Bonny Trans Niger Pipeline at Owokiri / Bodo City	2013	Rivers	16,000
Total	_	_	1,703,900

Source: UNDP (2006); Shell Nigeria (2018)

2.5 Impact of Petroleum Hydrocarbon in the Environment

In terms of organisms, they vary greatly in their sensitivity to petroleum hydrocarbons and predicting the environmental impacts of specific releases of a quantity of petroleum hydrocarbon requires much site specific information about the nature of the receiving body. Most of what is known about petroleum hydrocarbons comes from studies of catastrophic oil spills. Effects tend to reflect the amount of toxic hydrocarbons in the environment and the different susceptibility of organisms, population and ecosystems and doses are rarely directly proportional to the amount released; one must consider the type of petroleum hydrocarbons released and the susceptibility of the organisms due to the environmental processes acting on the released petroleum hydrocarbons. The toxicity to the organisms will depend on the available dose of petroleum available to an organism. When petroleum hydrocarbonsare released into the environment, processes alter the chemical composition of the petroleum hydrocarbon which alters the toxicity. Physical weathering may transform the petroleum hydrocarbons to a form less available to the organism. The chemical and physical properties of the petroleum hydrocarbon components determine the rate it passes into an organism. The bioavailability and persistence of specific hydrocarbons, the ability of an organism to accumulate and metabolize, fate of the metabolized products, metabolites of the hydrocarbon interphase with the normal metabolic process may alter an organism's chances of survival and reproduction in the environment. The narcotic effects of hydrocarbons on nerve transmission are the major biological factors in determining the ecological impacts of any release; other factors include photo - degradation and photo-activation. Birds and mammals are vulnerable to oil spills when their habitats become contaminated and this may reduce reproductive rates, survival and physiological impairment. In water, oil film floating on the water surface prevents natural aeration and leads to death of fresh water or marine life and on

land leads to retardation of vegetation growth, cause soil infertility for a long periods of time. Ayuba (2012) reported a study of the summary of some significant pollutants from the oil industry released into the environment as follows:

1. Exploration and Production activities include: drilling muds, cuttings, oil and greases, salinity, sulphides, turbidity, suspended solids, temperature, pH, heavy metals, biological oxygen demand (BOD_5) and chemical oxygen demand (COD).
2. Petroleum refining activities include: oil and greases, BOD_5, COD, phenol, cyanide, sulphide, suspended solids, toxic additives, hydrocarbons and total suspended solids.

Effects can be either direct damage of a resource or the ability of the environment to support a resource, an effect is only said to be over when complete recovery has taken place. To quantify the effects and recovery is difficult; damage to a small area containing highly valued resources can be of greater significance than damage to a much larger area devoid of valued resources. The US DOE has reported that the Niger Delta area has experienced 4,000 oil spill incidences since 1960. This has resulted in the loss mangrove trees due to the inability of the mangrove trees to withstand the high toxicity levels of the petrochemicals spilled into the habitat. The spills have also had adverse effects on the marine habitat which has become contaminated. This poses enormous human health risk from the consumption of contaminated seafood (Twumasi and Merem, 2006).The environmental problems of the Niger Delta result in generally land resource degradation, renewable resource degradation and environmental pollution, agricultural land degradation, fisheries depletion, deforestation, biodiversity loss, oil pollution, gas flaring and mangrove degradation (Ayuba, 2012).

2.6 Environmental Degradation in the Niger Delta and its Effect on the People

In terms of environmental changes occurring within the region, large areas of mangrove forest have been destroyed which is a major source of wood to the indigenous people. When oil spills occur, the oil spreads over a wide area affecting terrestrial and marine resources. Some past spills have necessitated the complete relocation of some communities, loss of ancestral homes, pollution of fresh water, loss of forest and agricultural land, destruction of fishing grounds and reduction of fish population, which is the major source of income for the Niger Delta people. All these constitute massive unquantifiable losses to farmers, fishermen and hunters. The pollution exposes people also to new risk of diseases (Ayuba, 2012).

2.7 Overview on Aromatic Hydrocarbons

2.7.1 Simple aromatic hydrocarbons

Simple aromatic compounds include the mono aromatic hydrocarbons, such as BTEX and phenol. These compounds are commonly present as ground water contaminants, due to spillages or leakages from fuel tanks and effluents from petroleum refineries and manufacturing units. They are acutely toxic and produce noticeable health effects upon exposure to concentrations higher than the EPA maximum contaminant levels. For example, benzene with a concentration higher than 0.005 mg/l may cause anemia and increase cancer risk; toluene with concentrations of 1 mg/l and above may damage the nervous system, kidney, and liver (Cao *et al.,* 2009).

2.7.2 Sources

The sources of aromatic hydrocarbon pollution are the treatment plants, which occur through the discharge of wastewater effluents and sludge, and contamination also results from

manufacture, handling, use, and disposal of these chemicals. The presence of these compounds in seawater could be as a result of the transport of contaminated wastewater effluent by rivers into oceans and seas. The government policies on environmental management have therefore mandated all industries to properly manage their waste. These policies include the treatment of wastewater generated by these industries before it comes in contact with natural water streams (Ayanda, 2014). Three major sources of PAHs are petrogenic, pyrogenic and biogenic.

2.7.3 Aromatic hydrocarbons in water

PAHs, one of the major groups of anthropogenic environmental pollutants, were firstly identified and measured in coastal waters, native mussels and fish of an industrialized South American estuary (Lawal, 2017). Penezić *et al.*, (2014) developed a new sensing system for PAHs in waters. The system consists of a wafer-based device with chip-based mercury on platinum microelectrode as a working electrode and a platinum auxiliary electrode, incorporated into a flow cell system with an external reference electrode. The mercury microelectrode was coated with a phospholipid-triglyceride mixed layer and interactions between anthracene, phenanthrene, pyrene and fluoranthene and the layer were monitored using rapid cyclic voltammetry. The layer proved sensitive to interactions with PAHs in 'organic - matter free' seawater, with respective detection limits of 0.33, 0.35, 0.15 and 0.32 μgl^{-1} for phenanthrene, pyrene, anthracene and fluoranthene. In surface waters, volatilization is the dominant fate process for xylenes. While biodegradation in water also occurs to some extent, it is likely of lesser significance as the estimated half-life for biodegradation of xylene in water (247.5 h is considerably greater than the half-life predicted for volatilization 5.6 h) (Alberta Environment, 2004).

2.7.4 Aromatic hydrocarbons in soil and sediment

Pollution pressures are always placed in the coastal and estuarine ecosystems of any developing and developed countries because of the elevated pollutants discharged from various sources such as industries, waste and human activities. PAHs in the environment were closely linked to human activities, which have been intensively studied for their geochemical interest as markers. Riverine inputs are the most important sources of PAHs in the coastal sediments and soil has been identified as the primary reservoir for PAHs in the United Kingdom. The waste generated from industrial processes and operations including domestic wastes when treated partially and disposed in soil –water environment enters to lakes, streams, rivers, oceans and other water bodies. The pollutants get dissolved or lie suspended in water or get deposited on soil sediment beds (Lawal, 2017). Aly Salem *et al*., (2014) analysed PAHs and aliphatic hydrocarbon in sediments collected from the Suez Gulf, Aqaba Gulf and the Red Sea proper stations, Egypt. Meanwhile, ΣPAH concentrations were in the range of 0.74 – 456.91 ng/g, with the mean value of 32.94 ng/g. The highest concentration of total PAHs is recorded in sediments collected from El-Quseir (456.91 ng/g), followed by that in Sharm El Mayaa (100.05 ng/g) and Suez 10 (97.19 ng/g), while lower concentrations are detected in Sheraton (0.74 ng/g), Ras Mohamed and Na'ama Bay (0.74, 6.86 and 11.1 ng/g, respectively). A general increasing trend for the relative retention of xylene in soil with increasing soil organic matter has been observed; however, the presence of other organic pollutants may result in competition for sorption sites and may increase the leaching of xylenes through the soil profile. Xylenes are expected to volatilize rapidly from moist and dry soil surfaces with an estimated half-life for all three isomers ranging from one-

minute to 2.2 days. Volatilization is the dominant fate process in soil (Alberta Environment, 2004).

2.7.5 Toxicokinetics of aromatic hydrocarbons

2.7.5.1 Absorption

Benzo (a) pyrene is rapidly absorbed after oral administration to rats. The extent of absorption of PAH from food is in the range of 20 – 50 %. The absorption is influenced by the composition of the diet such that the bioavailability from food increases with increasing lipid content (Larsen, 2013). In humans, xylene isomers are absorbed by the respiratory tract (60 – 65 %), gastrointestinal tract (up to 90 %) and skin (2 %) (Health Canada, 2014).

2.7.5.2 Distribution

Due to enterohepalitic Due to enterohepatic cycling, high levels can be found in the gastrointestinal tract irrespective of the route of administration. Studies in pregnant mice and rats have shown that PAH cross the placenta being detectable in the foetuses. Benzo [*a*] pyrene, dibenz [*a, c*] anthracene and chrysene were reported to be present in human milk and umbilical cord blood at low levels (Larsen, 2013). Limited information is available on the distribution of xylenes in humans and experimental animals following ingestion. Xylene isomers are relatively soluble in blood (Health Canada, 2014).

2.7.5.3 Metabolism

Metabolism and excretion has been studied in whole animals for anthracene, phenanthrene, pyrene, benz [*a*] anthracene, chrysene, benzo [*a*] pyrene, dibenz [*a,h*] anthracene, and 3 – methylcholanthrene. The general scheme of PAH metabolism involves oxidation to a range

of primary (epoxides, phenols, dihydrodiols) and secondary (diol epoxides, triols, tetrahydrotetrols, phenol epoxides) phase 1 metabolites catalysed by several different cytochrome P_{450} (CYP) species and other enzymes, followed by conjugation to phase 2 metabolites with glutathione, glucuronic acid or sulphate (Larsen, 2013). The metabolism of the three xylene isomers occurs primarily in the liver and to a lesser extent in the lung and kidneys. The main metabolic pathway that accounts for almost the entire absorbed dose of xylenes (90 %) in humans involves hydroxylation of a methyl group, which is catalyzed mainly by an isoform of CYP (CYP2E1), forming methylbenzyl alcohols (Health Canada, 2014).

2.7.5.4 Excretion

PAH metabolites are excreted in urine, bile and faeces. The bile is the major route of excretion accounting for 60 % of an intraveneous dose of benzo [a] pyrene whilst the urinary excretion was 3 %. The gastrointestinal microflora can hydrolyse glucuronic acid conjugates of PAH metabolites whereby the metabolites are released and can be re-absorbed, leading to enterohepatic cycling (Larsen, 2013). In humans, approximately 95 % of absorbed xylenes following inhalation are biotransformed and excreted as methylhippuric acids, while the remaining 5 % are eliminated unchanged in the exhaled breath. A small fraction (< 0.005 %) is eliminated unchanged in the urine, and < 2 % is eliminated as xylenols (Health Canada, 2014).

2.7.6 Toxicity, carcinogenicity and mutagenicity of aromatic hydrocarbons

The growing rate of industrialization is gradually leading to contamination and deterioration of the environment and pollution is likely to reach disturbing levels in the years ahead. PAHs are major pollutants and public concern over the deleterious effects of PAHs has grown

rapidly due to recognition of their toxicity, carcinogenicity, and teratogenicity. Benzo (a) pyrene (BaP) possesses the greatest carcinogenic potential among the various PAHs. PAHs are widespread in various ecosystems and are pollutants of great concern due to their potential toxicity, mutagenicity and carcinogenicity. Twelve PAHs likely carcinogenic (i.e., phenanthrene (Phe), anthracene (An), fluoranthene (Fluo), pyrene (Pyr), benz[*a*] anthracene (B[*a*] A), chrysene (Chry), benzo [*b*] fluoranthene (B[*b*] F), benzo [*k*] fluoranthene (B[*k*] F), benzo [*a*] pyrene (B[*a*] P), indeno [*1,2,3-cd*] pyrene (Ind), dibenz [*a, h*] anthracene (D [*a, h*] A), and benzo [*g, h, i*] perylene (B[*g,h*] P). Seven PAH compounds have been classified as probable human carcinogens: benz (*a*) anthracene, benzo (*a*) pyrene, benzo (*b*) fluoranthene, benzo (*k*) fluoranthene, chrysene, dibenz (*ah*) anthracene, and indeno (*1, 2, 3 - cd*) pyrene (Lawal, 2017). The most affected tissues were liver and kidney, although some effects have also been observed in the brain, heart and lung. Enlarged liver and kidney were observed in rats that were administered mixed xylenes by oral gavage for 90 consecutive days at doses as low as 750 mg/kg bw per day. Long-term animal studies have been carried out for xylenes. Overall, these studies did not support xylenes as tumour-inducing chemicals via the oral, inhalation or dermal route (Health Canada, 2014).

2.7.7 Ecotoxic effects of aromatic hydrocarbons

The toxicity of PAHs to aquatic organisms is affected by metabolism and photo-oxidation. They are generally more toxic in the presence of ultraviolet light. PAHs have moderate to high acute toxicity to aquatic life and birds. PAHs in soil are unlikely to exert toxic effects on terrestrial invertebrates, except when the soil is highly contaminated. Adverse effects on these organisms include tumors, reproduction, development, and immunity. Mammals can absorb PAHs by various routes e.g. inhalation, dermal contact, and ingestion. On the other hand, plants can absorb PAHs from soils through their roots and translocate them to other plant

parts. Uptake rates are generally governed by concentration, water solubility, and their physicochemical state as well as soil type. PAH-induced phytotoxic effects are rare. Full information and the database on this are still limited. Certain plants contain substances that can protect against PAH effects. Other plants can synthesize PAHs that act as growth hormones. PAHs are moderately persistent in the environment, and can be bio-accumulated. The concentrations of PAHs found in fish and shellfish are expected to be much higher than in the environment from which they were taken. Bioaccumulation has been also shown in terrestrial invertebrates. Nevertheless, metabolism of PAHs is sufficient to prevent biomagnifications (Abdel-Shafy and Mansour, 2016). Fugacity predictions indicate that most xylenes released to the environment will occur in the atmosphere and that volatilization is the dominant fate process (Alberta Environment, 2004).

2.7.8 Extraction

Generally, sample pre-treatment comprises: (1) solvent extraction of PAHs from filters and sorbents; (2) extract concentration; (3) some form of liquid, or solid, chromatography clean-up; (4) eluent concentration; and (5) injection into a gas chromatography (GC) for analysis (Lawal, 2017).

2.7.9 Detection and quantification of aromatic hydrocarbons

The detection and quantification of these compounds in environmental matrixes usually requires a pre-concentration or sample preparation step, prior to instrumental analysis by gas chromatography - mass spectrometer (GC - MS) or gas chromatography- flame ionization detector (GC - FID) (Ayanda, 2014).

2.7.10 PAH degradation techniques

2.7.10.1 Chemical degradation

Detecting the persistence of PAH after its degradation in environment is quite difficult. The existence of PAH in anaerobic condition depends on substrate interaction, pH and redox condition. Degradation of PAH in soil occurs through biotic process. Upon undergoing chemical reactions, PAHs are transformed into other derivatives of PAHs. As considerable amount of energy is required to change an aromatic compound into a non- aromatic compound, PAHs do not lose their aromaticity character. The efficiency of PAHs chemical degradation is limited because of their low aqueous solubility and vapour pressure. It has been reported that surfactants have the property to overcome the problems related to PAHs low aqueous solubility. Solubility of PAHs is enhanced in the presence of surfactants by decreasing the interfacial surface tension (Gupte *et al.*, 2016).

2.7.10.2 Bioremediation

Bioremediation involves the process of biodegradation and biotransformation where by organic contaminants are transformed or degraded to an environmentally safe levels in ground water and soil. The micro-organisms used for the biodegradation may be indigenous to a contaminated area or may be extraneous and brought to the contaminated site. The complete mineralization of the pollutants leads to the generation of CO_2, H_2O and biomass (Gupte *et al.*, 2016).

2.7.10.3 Phytodegradation

Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain or immobilize contaminants. There are various mechanisms like volatilization, rhizoremediation, phytotransformation, phytostabilization and hydraulic control that are used for degradation of pollutants from air, soil and water. Phytodegradation is also called

phytotransformation which involves degradation of organic contaminants to simple molecules or the incorporation of these molecules into plant tissues. It has been reported that the tall fescue grass (*Festuca arundinacea*) and switch grass (*Panicum virgatum*) have the ability to degrade 38 % of pyrene in 190 days (Gupte *et al.*, 2016).

2.7.10.4 Combined degradation

The usage of two or more degradation approaches is referred to as combined degradation and at present is considered as an approach for the removal of PAHs from contaminated sites. It has advantage over other degradation methods since it is regarded as efficient and cost-effective. Success in combined degradation of PAHs have been documented using pressurized assisted ozonation and integrated treatment using soil washing, ozonation and biological treatment of substrate. Combined degradation and phyto-degradation using inorganic nutrients with *E. crassipes* is an innovative approach which is gaining a wide acceptability (Gupte *et al.*, 2016).

2.8 Overview on Heavy Metals

2.8.1 Heavy metal related to oil spill

The rapid development of crude oil exploration and transportation increase the tendency to oil spill incident which released heavy metals as the major contaminants in the worldwide environment. Fish is an essential food for meet human nutrition. So, the presence of heavy metal in fish give impact to the human health. Furthermore, fish also can be good bioindicators of heavy metal contamination due to its capability to accumulate contaminants in tissue. Some authors reported that iron (Fe) recorded highest concentration in different parts of fish at Niger Delta. The study also related high level of the trace metal due to crude oil spill which is occurs regularly in the region. This occurrence is so harmful to human who

may consume the fish as their daily nutrient sources. Nigeria is the sixth largest petroleum producer in the world. The industry contributes approximately 80 % of nation income. Some other authors also reported that enrichment factors for strontium (Sr), zinc (Zn), lead (Pb), barium (Ba), and iron (Fe) were very high for every sample. The study summarizes that there is significant relationship between heavy metal pollution and crudeoil production industry which may be spillage have occurred in the process of production. This is very harmful because the high contamination of heavy metal is very dangerous to both aquatic environment and human health. Hence, it is important to determine concentrations of heavy metals in every difference level of environment in order to evaluate the possible risk of consumption. From the review conducted, Table 2.3 shows several studies on heavy metal related to oil spill conducted around the world. Based on the table, researchers tend to study heavy metal in many media such as sediment, water, seafood, plant, and crude oil itself to represent the level of contaminants in environment. The oil spill incident that involved in this study consistsof major and large scale spillage which causes devastated effect on aquatic environment (Ahmad *et al*., 2015).

Table 2.3: Significant heavy metal based on oil spill studies

Heavy Metal	Medium	Oil Spill Area
Zn, Cd, Ni, V, and Mn	Soil	Shengli Oilfield, China
Cr, Ni, Pb, and Zn	Sediment	Kachemak Bay, Exxon Valdez
V	Sediment	Mina Al Fahal, Oman
Cu and Zn	Water	Bohai Bay, China
Ni, Cd, Pb, and Cu	Mosses	Nigerian Petroleum Depot, Nigeria
Cd, Cr, Cu, Fe, Ni, and Pb	Water and Sediment	Niger Delta, Nigeria
Ni and V	Crude oil	Gulf of Mexico
V	Mussel	Prestige Oil Spill
Hg	Air	West Coast Korea
Zn, Cu, Cd, and Pb	Sediment	Gulf of Mexico
Pb, Ni, V	Oyster	Jiyeh Oil Spill
As, Cd, Cu, Pb, Hg, Ni, Sn, and Zn	Mussel	National Coastal Zone, United States
V	Mussel	Prestige Oil Spill
V and Ni	Sediment	Gulf of Thai
Pb, Zn, Cu, Ni, Cd, Co, Cr, Fe, and Mn	Fish	Niger Delta, Nigeria
Hg	Seabird	Prestige Oil Spill
Sr, Zn, Pb, Ba, and Fe	Soil, sediment, solid waste	Niger Delta, Nigeria
Zn, Cu, As, Pb, Mn, Mg, Se and V	Marine blue crab	Gulf War Oil Spill
Pb and Cd	Sediment	Gulf of Suez
Zn, V, Pb, and Cr	Seawater	Gulf War Oil Spill

Source: Ahmad *et al*. (2015)

2.8.2 Human health effect

In aquatic systems, the heavy metals of greatest concern are copper, zinc, cadmium, mercury and lead. These elements are toxic to organisms above specific threshold concentrations but many of them (e.g. copper and zinc) are essential for metabolism at lower concentrations. Lead and cadmium have no biological function. Other elements of concern are aluminium, chromium, selenium, silver, arsenic and antimony which have contributed to serious problems in freshwater, estuarine and coastal ecosystems (Ansari *et al.*, 2004).

Generally, lead (Pb) significantly appears as highest number of element determined throughout several studies related to oil spill conducted around the world. Pb becomes very harmful by entering into biota by several routes such as the inhalation of element in air, oral intake or ingestion in contaminated water, and is absorbed from food and soil in polluted areas. Subsequent to absorption, Pb is distributed evenly in human body via bloodstream and it is excreted at very slow rate through faeces and urine. Pb can be very dangerous to human health by causing multiple toxic effects. Higher than permissible level of exposure to Pb may lead to abnormalities such as alteration in haematological, immune, reproductive, nervous and renal systems.

Nickel (Ni) is a toxic element which human exposure to it by several pathways such as via inhalation, ingestion and dermal absorption. Nickel is present in different types of

environment such as soils and waters in form of soluble and insoluble compound. Many unique physical and chemical characteristics make this metal widely used in modern industry. Continous exposure to high level of nickel causes increased risk of lung cancer, nasal cancer, DNA damage, cell death, inflammation and impacts the cellular metabolism (Ahmad *et al*., 2015).

Since the 1960's, cadmium (Cd) was closely related to the 'itai-itai' painful bone disease due to high level in Japan environment. The contamination was caused by mining activities that caused contaminant transport into river and remained in paddy fields before being consumed by local people. The element has also recently been shown to be an endocrine-disrupting chemical with estrogenic properties and a potential prostate carcinogen. Naturally, Cd accumulated with high level in the kidney which can be persistent and toxic to human body. According to some reseachers, inorganic Cd is very toxic to humans and can enter the human body through inhalation and ingestion. The high concentration of Cd closely related to nausea, vomiting, diarrhea, headache, abdominal pain and also has resulted in death within 1-2 weeks subsequently by liver and kidney damage. A study conducted at Europe summarized that significant correlation between cancer incidence and Cd concentration in topsoil and stream water. Excessive zinc intake can cause acute and chronic toxicity. Acute toxicity of high zinc intake causes several adverse effects such as nausea, loss of appetite, vomiting, diarrhea, headaches and abdominal cramps. Zinc gluconate is one of the elemental zinc examples that caused nausea and vomiting if high intakes occur in humanbody. High intakes of zinc also altered or reduced another process function such as iron function, immune function, and low copper status (Ahmad *et al*., 2015). Arsenic is toxic to human beings, especially the trivalent compounds (As^{3+}). In low doses, arsenic is used as a medication to enhance growth. At low intake levels, arsenic can accumulate in the body over time. Trivalent chromium (Cr^{3+}) may be essential in human nutrition, but hexa-valent chromium

(Cr^{+6}) is highly toxic. Intake of hexa-valent chromium can cause hemorrhaging in the liver, kidneys, and respiratory organs. When people are exposed to hexavalent chromium, dermatitis and ulceration and perforation of the nasal septum have been developed. Also, gastric cancers, presumably from excessive inhalation of dust containing chromium, have been reported. Copper is essential for life due to its major role in enzyme functions. Copper in large amounts is quite toxic. For example, copper salts are used to kill bacteria, fungi, and algae, and paints containing copper are used on ship hulls to prevent fouling by marine organisms. Acute exposure to overdose causes an immediate metallic taste, followed by epigastric burning, nausea, vomiting, and diarrhea (Coung, 2005). Mercury effects on humans include ataxia, attention deficit, blindness, deafness, decreased rate of fertility, dementia, dizziness, dysphasia, gastrointestinal irritation, gingivitis,kidney problem, loss of memory, pulmonary edema, reduced immunity, sclerosis (Ayangbero and Babalola, 2017). In humans, haemoglobin binds oxygen in a reversible way and thus assures the transport of oxygen from the lungs to the tissues. The highest concentrations of iron are in the liver, spleen, then kidney and heart. Iron deficiency leads to a sort of anaemia, causing fatigue, headache and anorexia. On the other hand, intake of more than 0.5 g of soluble iron salts may cause grave injury to the alimentary canal followed by a series of serious effects such as hepatitis. Continued intake of excessively high amounts of iron causes haemochromatosis which eventually leads to liver cirrhosis (Ansari *et al*., 2004).

2.8.3 Bioremediation of heavy metals by microorganisms

Bioremediation is an innovative technique for the removal and recovery of heavy metal ions from polluted areas, and involves using living organisms to reduce and/or recover heavy metal pollutants into less hazardous forms, using the activities of algae, bacteria, fungi, or plants. It has been employed for the removal of heavy metals from contaminated wastewaters

and soils. This method is an appealing alternative to physical and chemical techniques, and the use of microorganisms plays a significant role in heavy metal remediation. Detoxification can occur through the valence transformation mechanism. This is particularly applicable in the case of metals whose different valence states vary in toxicity. In mercury-resistant bacteria, organomercurial lyase converts methyl mercury to Hg (II), which is one hundred-fold less toxic than methyl mercury. The reduction of Cr (VI) to Cr (III) is widely studied, with Cr (III) having less mobility and toxicity. Other detoxification mechanisms of heavy metals are accomplished through metal binding, vacuole compartmentalization, and volatilization. Metal binding involves chelators, such as metallothein, glutathione – derived-peptides called phytochelatin, and metal binding peptides. These chelators bind to heavy metals and facilitate microbial absorption and the transportation of metal ions. Volatilization mechanisms involve turning metal ions into a volatile state. This is only possible with selenium (Se) and mercury (Hg), which have volatile states. Mercury-resistant bacteria utilize the MerA enzyme to reduce Hg (II) to the volatile form Hg (0). The reduction of Se (V) to elemental Se (0) has been employed to remediate contaminated waters and soils. The metabolic processes of these organisms help to transform pollutants in the environment. Biosorption, bioaccumulation, biotransformation, and biomineralization are the techniques employed by microorganisms for their continued existence in metal polluted environment. These strategies have been exploited for remediation procedures (Ayangbero and Babalola, 2017).

2.9 Microbial Tests

Microbial toxicity tests are known to be fast, simple, and inexpensive. These properties of the tests have resulted in their ever-increasing use in environmental control, assessment of pollutants in waste, and so on. Toxicity test methods based on the reaction of microbes are

useful in toxicity. Microbial tests can be performed using a pure culture of well - defined single species or a mixture of microbes. In particular, they can be a very valuable tool for the toxicity assessment and classification of samples as shown in Figure 2.1.

The variables measured in microbial toxicity assays may be lethality, growth rate, change in species diversity, decrease in degradation activity, and energy metabolism or activity of specific enzymes. The results are generally expressed as the dose-response concentration and the EC_{50} or EC_{10} values are determined(Selivanovskaya *et al*., 2006). The following microbial tests are discussed below:

2.9.1 Marine algal growth inhibition test

The Marine Algaltoxkit makes use of microalgae inoculum that can be stored for several months without losing its viability. Transfer of the algal inoculum in an adequate growth medium reactivates the microalgae leading - within 3 days – to a culture in the exponential growth phase ready for the bioassay. Optical density measurements of the algal suspensions and toxicant volumes at 670 nm wavelength in 10 cm long cells correlate very well with algal numbers and are hence in accordance with the prescription of ISO Guideline 10253 and other standard methods for determination of algal densities. A 72 h algal growth inhibition test is performed in long cell test vials, with the marine diatom *Phaeodactylum tricornutum*. The Marine Algaltoxkit test has been modelled on and follows the prescriptions of the ISO guideline "Water Quality - Marine Algal Growth Inhibition Tests with *Skeletonema costatum*and *Phaeodactylum tricornutum*" (Guideline ISO/CD 10253) (EBPI, 2016).

2.9.2 Phytotoxicity test

The germination index (GI) gives an idea of the effect of contamination on both seed germination and root growth. It's used to study the dynamics of early plant growth in function of the concentration of the spiked chemical compound(s). It measures the decrease (or the absence) of germination and of the growth of the young roots after a few days of exposure of seeds of selected higher plants to toxicants or to contaminated soils, in comparison to the germination and growth of the controls in reference soil according to the procedure described in detail in the Phytotoxkit Standard Operational Procedure. After incubation at 25 ± 2 °C for 3 - 5 days, the number of germinated seeds and the length of each root were used to determine the germination index (Emami *et al.*, 2014; EBPI, 2016).

2.9.3 *Artemia* toxicity screening test

The Artemia toxicity test-kitcontains all the materials to perform standardized, simple and cost-effective bioassays for screening toxicity in estuarine (brackish) water and seawater. Using instar II-III larvae of the brine shrimp *Artemia franciscana* hatched from cysts, an acute toxicity test is executed in 24 h. Each ARTOXKIT M provides for 6 complete tests (range finding or definitive 24 h LC_{50}), or 5 bioassays and 1 quality control test with a reference toxicant (EBPI, 2016).

2.9.4 Toxi - chromo test

The Toxi-chromo test™ can be employed for qualitative measurements as well as experiments to quantify toxic potency. The assay is based on the ability of substances (toxicants) to inhibit the *de novo* synthesis of an inducible *β*-galactosidase enzyme in a highly permeable mutant of *Escherichia coli*. Addition of a chromogen after an exposure period produces an easy to read colorimetric endpoint that measures *β*-galactosidase activity in the affected bacteria. Results can be interpreted qualitatively using the naked eye or

quantitatively with a spectrophotometer and the assay can be customized to meet client laboratory specifications. Comparing the amount of colour produced between a test sample and a reference sample provides a measure of toxic potency (EBPI, 2016).

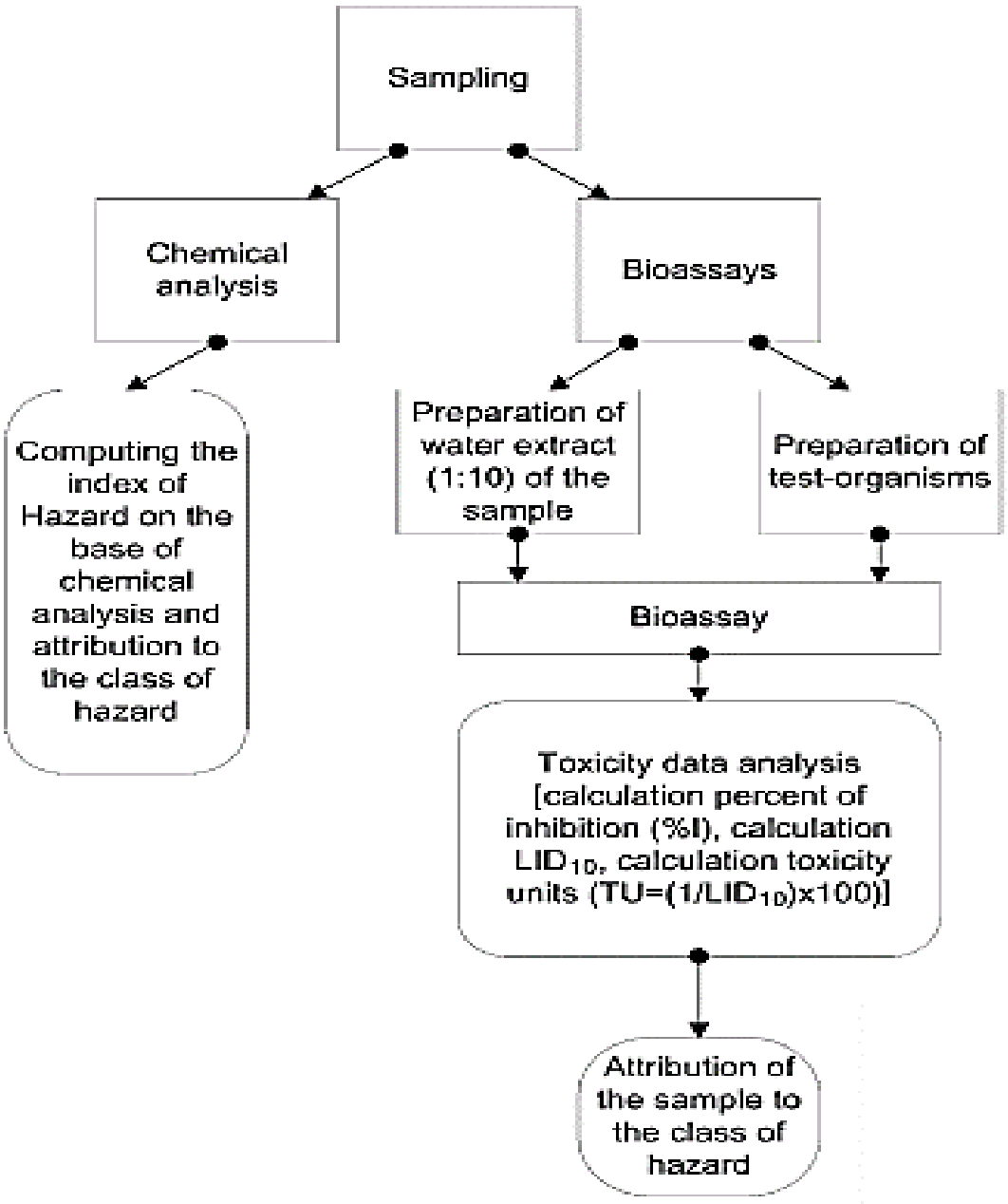


Figure 2.1: Proposed ecotoxicological procedure for assessment of solid waste toxicity and calculation of the classes of hazard
Source: Selivanovskaya *et al*. (2006)

2.10 Microbial Degradation

Monitoring and removing xylene from the environment has been viewed as a high priority around the world. Physical, chemical and biological approaches can be used to remove xylene and other petroleum hydrocarbons from contaminated sites. Microbial biodegradation is less expensive and hence considered one of the most promising alternative methods for cleaning up the environment from petroleum hydrocarbons (Irshaid and Jacob, 2015).

In recent decades, the presence of petroleum utilizing bacteria in oil and gas fields has been reported in several regions of the world. These include xylene - degrading microorganisms such as *Bacillus stearothermophilus*, *Arthrobacter*, *Acinetobacter calcoaceticus*, *Pseudomonas putida*, *Pantoea agglomerans* and *Enterobacter cloacae*.Several studies have reported that the growth rates of aromatic hydrocarbon - degrading bacteria were affected by the concentration of xylene compounds, temperature, pH of the medium and other factors (Irshaid and Jacob, 2015). Some studies showed *Pseudomonas* can adapt to diverse substrates and possess several catabolic pathways capable of acting on recalcitrant substances. Some studies with *Pseudomonas putida* identified metabolic pathways dedicated to gasoline components, like benzene, toluene and xylene (Otenio *et al.,* 2005). Figure 2.2 shows the degradation of BTEX compounds by *Pseudomonasputida.*

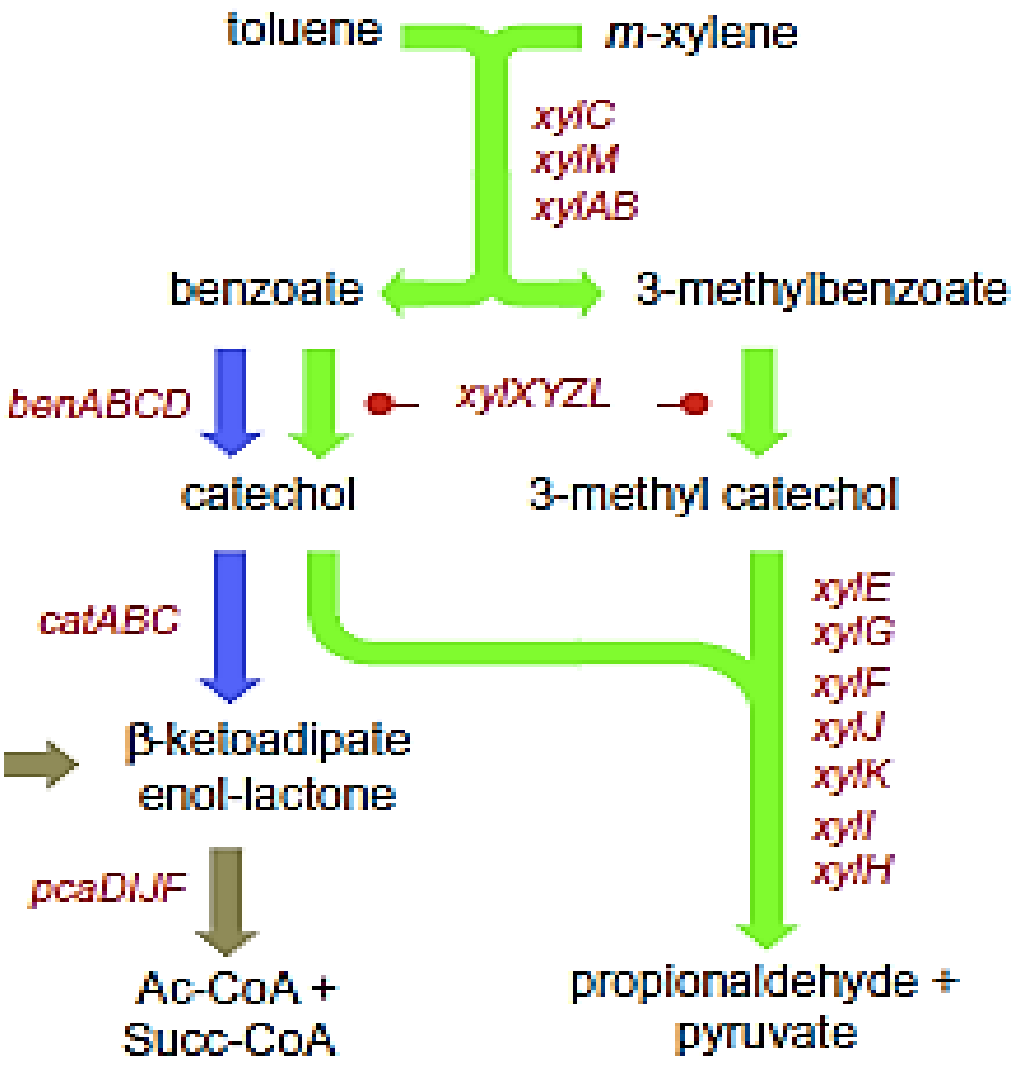

toluene
m-xylene
xylC
xylM
xylAB
benzoate
3-methylbenzoate
benABCD
xylXYZL
catechol
3-methyl catechol
catABC
xylE
xylG
xylF
xylJ
xylK
xylI
xylH
β-ketoadipate
enol-lactone
pcaDIJF
Ac-CoA +
Succ-CoA
propionaldehyde +
pyruvate

Figure 2.2: Aromatic catabolic pathways sharing metabolites in *P. putida* mt2. Key reactions connected to benzoate and β – ketoadipate catabolism
Source: Kim *et al*. (2015)

Anthracene is a tricyclic aromatic hydrocarbon, found widely in the environment. It has been a model substrate for various studies on degradation of PAHs. Anthracene is oxidised in the positions 1, 2 and gets converted to cis-1, 2 – dihydroxy-1, 2-dihydroanthracene. It is further converted to 1, 2-dihydroxyanthracene which uses NAD^+ dependent dihydrodiol dehydrogenase. Moreover, oxidation of 1, 2-dihydroxyanthracene undergoes ring fission to form a cis – 4-(2 – dihydroxynaphth – 3-yl) – 2 – oxobut-enoic acid as a product. This product gets converted to 2-hydroxynaphthoic acid. Further, the fission helps the metabolic product to form salicylate and catechol through 2, 3-dihydroxynapthalene. The schematic proposed pathway for anthracene degradation is shown inthe Figure 2.3.

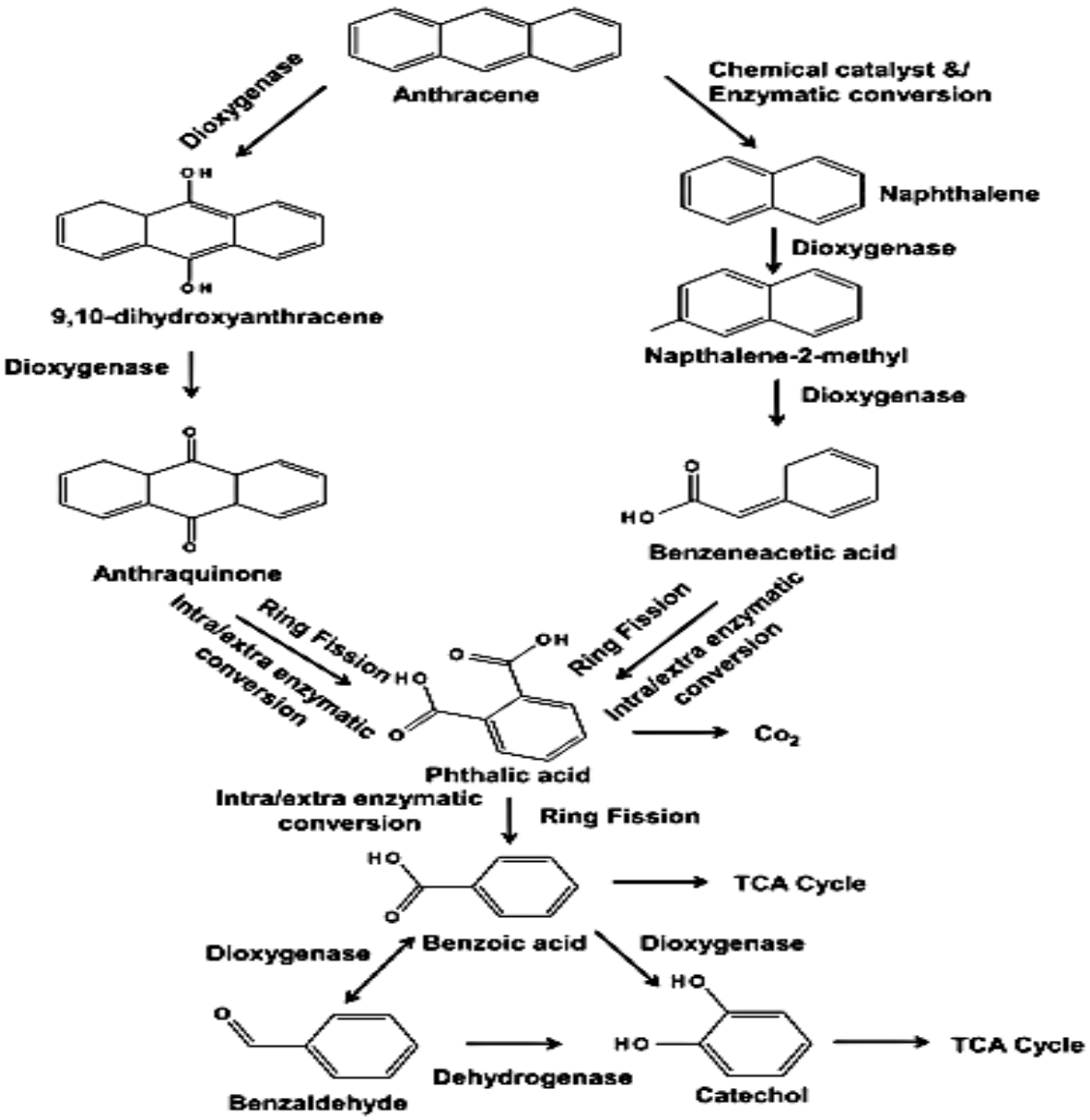

Anthracene
Dioxygenase
Chemical catalyst &/
Enzymatic conversion
9,10-dihydroxyanthracene
Naphthalene
Dioxygenase
Napthalene-2-methyl
Dioxygenase
Dioxygenase
Anthraquinone
Benzeneacetic acid
Ring Fission
Intra/extra enzymatic
conversion
Ring Fission
Intra/extra enzymatic
conversion
Phthalic acid
Co2
Intra/extra enzymatic
conversion
Ring Fission
TCA Cycle
Dioxygenase
Benzoic acid
Dioxygenase
Benzaldehyde
Dehydrogenase
Catechol
TCA Cycle

Figure 2.3: Proposed anthracene degradation pathway exhibited by marine *Bacillus licheniformis* MTCC 5514
Source: Swaathy *et al.* (2014a)

Pyrene possessing four benzene rings is a byproduct of gasification processes and other incomplete combustion processes. Many bacterial isolates capable of degrading pyrene have been studied. *Mycobacterium* as Gram-positive species has been most widely studied for degrading pyrene by using it as a sole carbon and energy source. *Mycobacterium* spp. are known to have high cell surface hydrophobicity and adhere to the emulsified solvent droplets. Other pyrene degrading strains isolated include *Rhodococcus* sp., *Bacillus cereus*, *Burkholderia cepacia*, *Cycloclasticus* sp. P1, *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Sphingomonas* sp. VKM B-2434, *Sphingomonas paucimobilis*, and *Stenotrophomonas maltophilia*. The schematic proposed pathway for pyrene degradation is exhibited in the Figure 2.4.

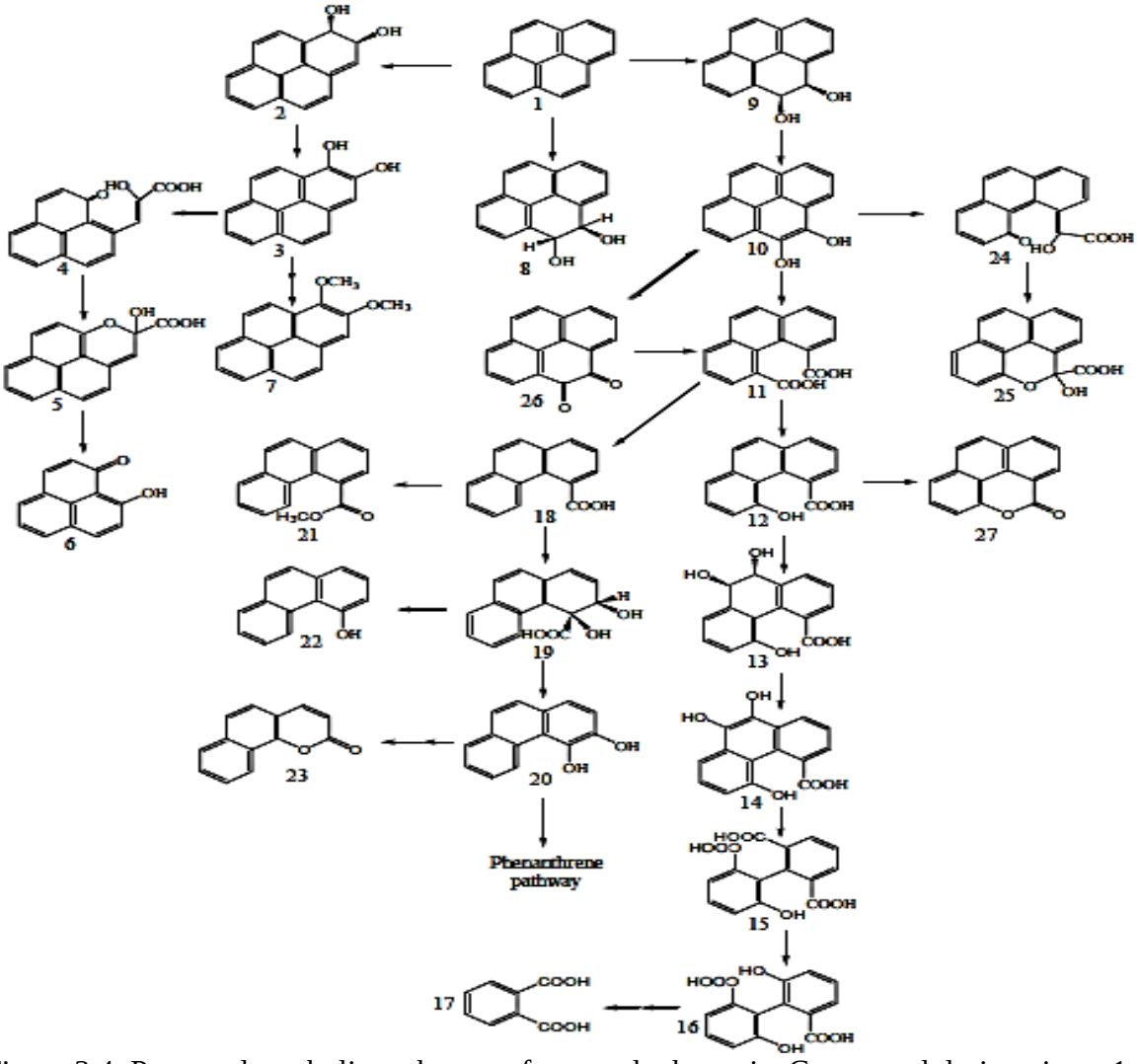


Figure 2.4: Proposed catabolic pathways of pyrene by bacteria. Compound designations: 1, pyrene; 2, pyrene – *cis* -1, 2 - dihydrodiol; 3, pyrene - 1,2 - diol; 4, 2 -hydroxy - 3-(perinaphthenone – 9 - yl) - propenic acid; 5, 2 – hydroxyl - 2*H* – 1 – oxa – pyrene – 2 - carboxylic acid; 6, 4 - hydroxyperinaphthenone; 7, 1, 2 - dimethoxypyrene; 8, pyrene – *trans* - 4,5 - dihydrodiol; 9, pyrene – *cis* - 4,5 - dihydrodiol; 10, pyrene - 4,5 - diol; 11, phenanthrene - 4, 5 - dicarboxylic acid; 12, 4 carboxyphenanthrene – 5 - ol; 13, 4 – carboxy -

5-hydroxy – phenanthrene - 9,10 - dihydrodiol; 14, 4 – carboxyphenanthrene - 5, 9, 10 - triol;15, 2, 6, 6'-tricarboxy - 2' - hydroxybiphenyl; 16, 2, 2'- dicarboxy - 6, 6'- dihydroxybiphenyl; 17, phthalic acid; 18, 4 - phenantroic acid; 19, 3, 4 – dihydroxy - 3, 4 – dihydro – phenanthrene – 4 - carboxylic acid; 20 phenanthrene - 3, 4 - diol; 21, 4 - phenanthroic acid methyl ester; 22, 4 -hydroxyphenanthrene; 23. 7, 8 - benzocoumarin; 24, 2 – hydroxyl – 2 - (phenanthrene – 5 – one – 4 - enyl) - acetic acid; 25, 5 – hydroxyl - 5*H* – 4 – oxapyrene – 5 - carboxylic acid; 26, pyrene - 4,5 - dione; 27, 4 – oxa – pyrene – 5 - one. Source: Seo *et al.* (2009)

2.10.1 Genetic aspects of aromatic petroleum hydrocarbon degradation

Several new methodologies have enabled recent studies on the microbial biodegradation mechanisms of organic pollutants. Culture-independent techniques for analysis of the genetic and metabolic potential of natural and model microbial communities that degrade organic pollutants have identified new metabolic pathways and enzymes for aerobic and anaerobic degradation of aromatic – ring compounds as shown in Figure 2.5. According to several authors, both dioxygenase and monooxygenase enzymes were considered as major degrading enzymes in the degradation of PAHs. These genes are important genes responsible for catabolizing low molecular weight as well as high molecular weight PAHs. Some also observed theformation of anthrone by alkaliphilic bacteria at C9 andC10 positions and further leads to the formation of quinone productof PAHs. According to some authors, anthraquinoneis the common oxidation product of PAH degradation. Ringopening of anthraquinone molecule by the presence of extracellularand intracellular enzyme systems generates phthalic acid,which, further undergoes ring fission reaction by the enzymaticsystem releasing benzoic acid which is then transformed to benzaldehyde,and thereafter converted to catechol by the same enzymesystem. According to some others,similar to naphthalene degradation pathway, catechol is alsodegraded to simple aliphatic compounds. Though naphthalenehas been identified as one of the degraded products in the presentstudy, the

presence of di-hydroxy anthracene and anthraquinonereveals that the catabolism has been realized through dioxygenasesystem of the isolate (Swaathy *et al*., 2014a).

Genetic factors play important roles in conferring biodegradation potentials on microorganisms. Plasmids probably play leading role in this aspect. The ability to degrade more recalcitrant components of petroleum such, as the aromatic fractions are generally plasmid mediated. Exposures of a microbial community to hydrocarbons have been shown to result in an increase in the numberof bacterial plasmid types as presented in Table 2.4. Catabolic plasmids are non-essential genetic elements in so far as viability and reproduction of an organism is concerned,but they do provide a metabolic versatility not normally present in the cell. Such genetic potential allows for the evolution of integrated and regulated pathways for thedegradation of hydrocarbons. The observed increase in the study of the genetics of such systems has closely paralleled the development of advances in molecularbiology, particularly the application of recombinant DNA technology, gene probes, and polymerase chain reaction (PCR) technology. Many bacterial catabolic pathways are specified by conjugative plasmids (Okoh, 2006). These plasmids are readily transferred laterally into new host bacteria, thereby enhancing the metabolic potential of other members of an ecosystem. Conjugative plasmids are thus important agents of genetic changes and evolution in bacteria, and could be picked up from or brought together in different organisms as groups of genes, which through mutations and recombination can specify new metabolic functions. For example, five aerobic toluene degradative pathways are characterized in pseudomonads. The best characterized of these pathways is encoded by the TOL plasmid (pWW0) of *P. putida* PaW1, which converts toluene to benzyl alcohol, benzaldehyde, benzoate, and catechol, which further undergoes *meta* cleavage by an extradiol dioxygenase, or catechol 2,3-dioxygenase (*C230*). *Pseudomonas putida* F1 metabolizes toluene to 3-

methylcatechol, which undergoes *meta*-cleavage by a *C230*. Three other pathways for the catabolism of toluene were observed in *Burkholderia cepacia* G4, *P. pickettii* PK01 and *P.mendocina* KR1, in which toluene is respectively converted to *ortho*-, *meta*-, and *para*-cresol. Peripheral oxygenases of these five toluene degradative pathways possessed well-distinguished substrate specificity. Particularly, xylenes can only be degraded by the *xyl* pathway encoded by TOL plasmid (Okoh, 2006).

In an environment rich in a particular organic compound, a selective pressure may lead to acquisition and maintenance of a plasmid that specifies a corresponding catabolic pathway. Many degraders of exotic compounds have been isolated from soil or water contaminated with such compounds. In some cases, the same catabolic genes may be located on a plasmid in one organism and on the chromosome in another, and these catabolic genes may influence the expression of other set of catabolic genes present in the same cell. In at least one instance of the TOL plasmid, transposition of catabolic genes from the plasmid to the chromosome has been demonstrated in the laboratory (Okoh, 2006).

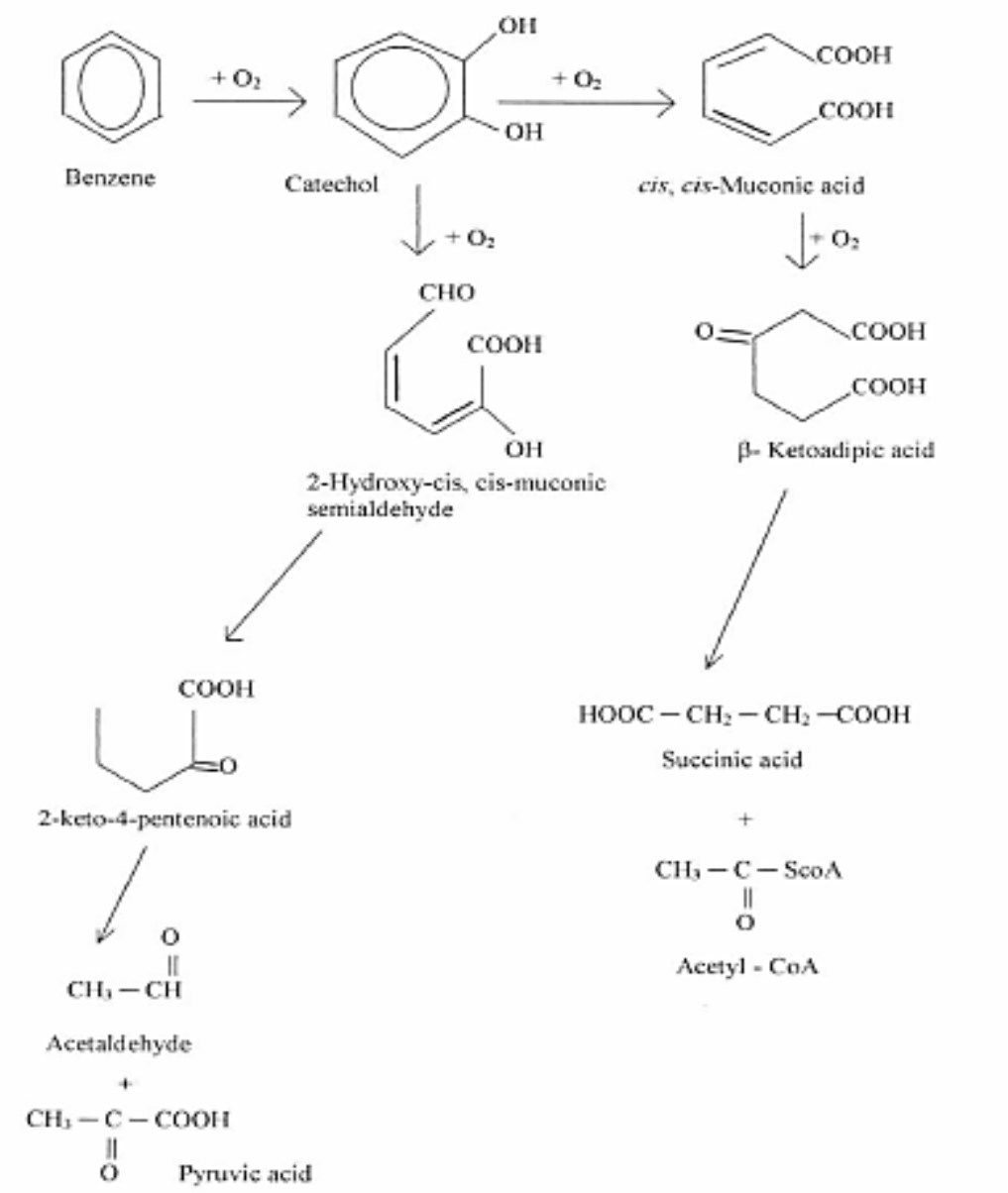

Benzene
+ O2
Catechol
OH
OH
+ O2
cis, cis-Muconic acid
COOH
COOH
+ O2
2-Hydroxy-cis, cis-muconic semialdehyde
CHO
COOH
OH
+ O2
β- Ketoadipic acid
O
COOH
COOH
2-keto-4-pentenoic acid
COOH
O
Acetaldehyde
CH3 — CH
O
Pyruvic acid
CH3 — C — COOH
O
Succinic acid
HOOC — CH2 — CH2 —COOH
+
Acetyl - CoA
CH3 — C — ScoA
O

Figure 2.5: Microbial metabolism of the aromatic ring by *meta*- or *ortho*- cleavage as shown for benzene
Source: Okoh (2006)

Table 2.4: Plasmids encoding catabolic functions

Plasmid	Host	Component(s) catabolised
TOL	*Pseudomonas putida*	Toluene, *p*- and *m*-xylene
NAH	*Pseudomonas putida*	Naphthalene
SAL	*Pseudomonas putida*	Salicylate
pND50	*Pseudomonas putida*	*p* - Cresol
pWW31	*Pseudomonas putida*	Phenylacetate
pJP1	*Alcaligenes paradoxa*	2,3 - Dichlorophenoxyacetic acid
pJP4	*Alcaligenes eutrophus*	2,4 - Dichlorophenoxyacetate & 3-chlorobenzoate
pKF1	*Acinetobacter* sp.	4 – Chlorobiphenol
pAC21	*Pseudomonas* sp.	4 – Chlorobiphenol
pRE1	*Pseudomonas putida*	Isopropyl benzene
pCIT1	*Pseudomonas* sp.	Aniline
pAC25	*Pseudomonas putida*	3 – Chlorobenzoate
pWR1	*Pseudomonas* sp.	3 – Chlorobenzoate
pCS1	*Pseudomonas putida*	Parathion

Source: Okoh (2006)

2.11 Properties and classification of surfactants

2.11.1 Properties

The unique and distinct properties of biosurfactants when compared to their chemically synthesized counterparts and broad substrate availability made them suitable for commercial applications. The major distinctive features of each property of biosurfactants are discussed below:

2.11.1.1 Surface and interface activity

Surfactant helps in reducing surface tension and the interfacial tension. Surfactin produced by *B. subtilis* can reduce surface tension of water to 25 mN m^{-1} and interfacial tension water/hexadecane to less than 1 mN m^{-1}. In general, biosurfactants are more effective and efficient and their Critical Micelle Concentration (CMC) is about several times lower than chemical surfactants i.e. for maximal decrease on surface tension, less surfactant is necessary (Vijayakumar and Saravanan, 2015).

2.11.1.2 Temperature and pH tolerance

Most of the biosurfactants and their surface activity are resistant towards environmental factors such as temperature and pH. Some authors reported that lichenysin from *Bacillus licheniformis* was found to be resistant to temperature up to 50 °C, pH between 4.5 and 9.0 and NaCl and Ca concentrations up to 50 and 25 g l^{-1}, respectively. Since industrial processes involve exposure to extremes of temperature, pH and pressure, it is necessary to isolate novel microbial products that are able to function under these conditions (Vijayakumar and Saravanan, 2015).

2.11.1.3 Biodegradability

Microbial derived compounds can be easily degraded when compared to synthetic surfactants and are suitable for environmental applications such as bioremediation/biosorption. The increasing environmental concern forces us to search for alternative products such as biosurfactants. Synthetic chemical surfactants impose environmental problems and hence, biodegradable biosurfactants from marine microorganisms were concerned for the biosorption of poorly soluble polycyclic aromatic hydrocarbons, phenanthrene contaminated in aquatic surfaces (Vijayakumar and Saravanan, 2015).

2.11.1.4Low toxicity

Although, very few literatures were available regarding the toxicity of biosurfactants, they are generally considered low or non-toxic products and are appropriate for pharmaceutical, cosmetic and food uses. The low toxicity profile of biosurfactants, sophorolipids from *Candida bombicola* made them useful in food industries (Vijayakumar and Saravanan, 2015).

2.11.1.5 Emulsion forming and emulsion breaking

Biosurfactants may act as emulsifiers or de-emulsifiers. Emulsions are generally two types: oil – in-water (o/w) or water – in-oil (w/o) emulsions. They possess a minimal stability which may be stabilized by additives such as biosurfactants and can be maintained as stable emulsions for months to years. Liposan is a water-soluble emulsifier synthesized by *Candida lipolytica* which has been used to emulsify edible oils by coating droplets of oil, thus forming stable emulsions (Vijayakumar and Saravanan, 2015).

2.11.1.6 Antiadhesive agents

A biofilm can be described as a group of bacteria/other organic matter that have colonized/accumulated on any surface. The first step on biofilm establishment is bacterial adherence over the surface was affected by various factors including type of microorganism, hydrophobicity and electrical charges of surfaces, environmental conditions and ability of microorganisms to produce extracellular polymers that help cells to anchor to surfaces. The biosurfactants can be used in altering the hydrophobicity of the surface which, in turn, affects the adhesion of microbes over the surface (Vijayakumar and Saravanan, 2015).

2.11.2 Biosurfactant classification

Unlike chemically synthesized surfactants, which are classified according to the nature of their polar grouping, biosurfactants are categorized mainly by their chemical composition and their microbial origin into five categories namely: glycolipids, lipopeptides, fatty acids, polymer type and particulate biosurfactants (Gharaei-Fathabad, 2011).

2.11.2.1 Glycolipids

Most known biosurfactants are glycolipids. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids (Gharaei-Fathabad, 2011).

2.11.2.2 Lipopeptides and lipoproteins

A large number of cyclic lipopetides including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) possess remarkable surface-active properties (Gharaei-Fathabad, 2011).

2.11.2.3 Fatty acids, phospholipids and neutral lipids

Several bacteria and yeasts produce large quantities of fatty acids and phospholipid surfactants during growth on n-alkanes (Gharaei-Fathabad, 2011).

2.11.2.4 Polymeric biosurfactants

The best studied polymeric biosurfactants are emulsan, liposan, mannoprotein and other polysaccharide-protein complexes (Gharaei-Fathabad, 2011).

2.11.2.5 Particulate biosurfactants

Extracellular membrane vesicles partition hydrocarbons to form a microemulsion which plays an important role in alkane uptake by microbial cells (Gharaei-Fathabad, 2011).

2.11.3 Potential applications of biosurfactants in industries

These molecules have an unlimited number of uses that involves every industry and every aspect of life: oil industry, pharmaceuticals, testing quality of condoms, agriculture, hygiene

and cosmetic, cement, beer and beverages, textiles, paint, detergents and cleaning and food processing. However, the applications depend on applied properties and the mechanism of action (Gharaei-Fathabad, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Sampling Sites

The studied areas were Abonema Wharf Water Front (Plate 3.1) in Akuku-Toru Local Government Area within Rivers West senatorial district, Nembe Water-side (Plate 3.2) in Port Harcourt Local Government Area within Rivers East senatorial district, and Onne Light Flow Terminal Seaport (Plate 3.3) located in Eleme Local Government Area of Rivers State within Rivers South – East senatorial district. Abonema town is 53 km and Abonema Wharf Water Front is 3 -5 km from Port Harcourt capital city; Nembe water side is located within Port Harcourt capital city of Rivers State, while Onne Light Flow Terminal is about 35 km east from Port Harcourt capital city of Rivers State and 7 km from Onne town. These sites were geo-referenced using Handheld Global Positioning System (GPS) GPSMAP 76sc with the coordinates obtained from the sampling points or positions (Appendices Ia - c). The coordinates were later used to download the Geoeye Satellite Images from the online archive of digital globe. Thereafter, the images were Georeferenced in ILWIS Software version 3.30

and exported to ArcGis 10.20 where the imageries were digitised to produce thematic maps indicating the land use and land cover of different sampling points of the three studied areas (Plates 17, 18 and 19).Abonema Wharf Water Front, Nembe Water-side and Onne Light Flow Terminal Seaport were located between latitude 4°46'15.82"N to latitude 4°46'38.01"N and longitude 7°0'0.54"E to longitude 7°0'34.82"E with average elevation of 4.1 m, latitude 4°45'8.72"N to latitude 4°45'26.42"N and longitude 7°1'11.37"Eto longitude 7° 2'14.54"Ewith average elevation of 2.7 m and latitude 4°41'32.58"N and 4°41'58.18"N and longitude 7°9'26.34"E and 7°10'48.82"Ewith average elevation of 2.3 m, respectively. The sampling sites were selected beacause they are the major locations in the three senatorial districts exposed tohuman-induced pressures resulting from urbanization, industrialization and intensive navigation across Rivers State marine environment. Abonema Wharf Water Front community (Appendices Id and Ig) is a popular and busy commercial but dangerous jetty area close to Portharcourt city inhabiting tens of thousands of different families living close to petroleum tank farms and tankers queue up daily to load refined petroleum products. Nembe Waterside (Appendices Ie and Ih) is situated very close to Creek road market, Port Harcourt, Nigeria. It shares boundary with Bayelsa and links Port Harcourt city with Bonny Island where most of the oil installations in Rivers State are. It also links the Island directly with the Atlantic Ocean through which crude oil is exported by massive oil tankers. Onne Light Flow Terminal Seaport (Appendices If and Ii) is a port of Nigeria and the largest oil and gas free zone in the world supporting exploration and production for Nigerian activities. It is situated on the Bonny River Estuary along Ogu creek and account for over 65 % of the export cargo through the Nigerian Sea Port. Anthropological survey revealed the presence of human activities such as transportation of petrochemical products through tankers, canoes, boats and ships to neighboring villages, towns, cities, states and nations due to the presence of multinational petrochemical and oil servicing industries such as Chevron Nigeria Limited,

Cameron Offshore services, Exxon Mobil Nigeria Limited, Socotherm Pipecoaters, Baker Hughes Oil Servicing Company, Aiteo Energy Resource, Sorelink Oil and Dozzy Oil and Gas and others that generate the wastes that contaminate the sites.

Plate 3.1: Geoeye satellite image (2016) showing the Abonema sample points
Source: DigitalGlobe (2018)

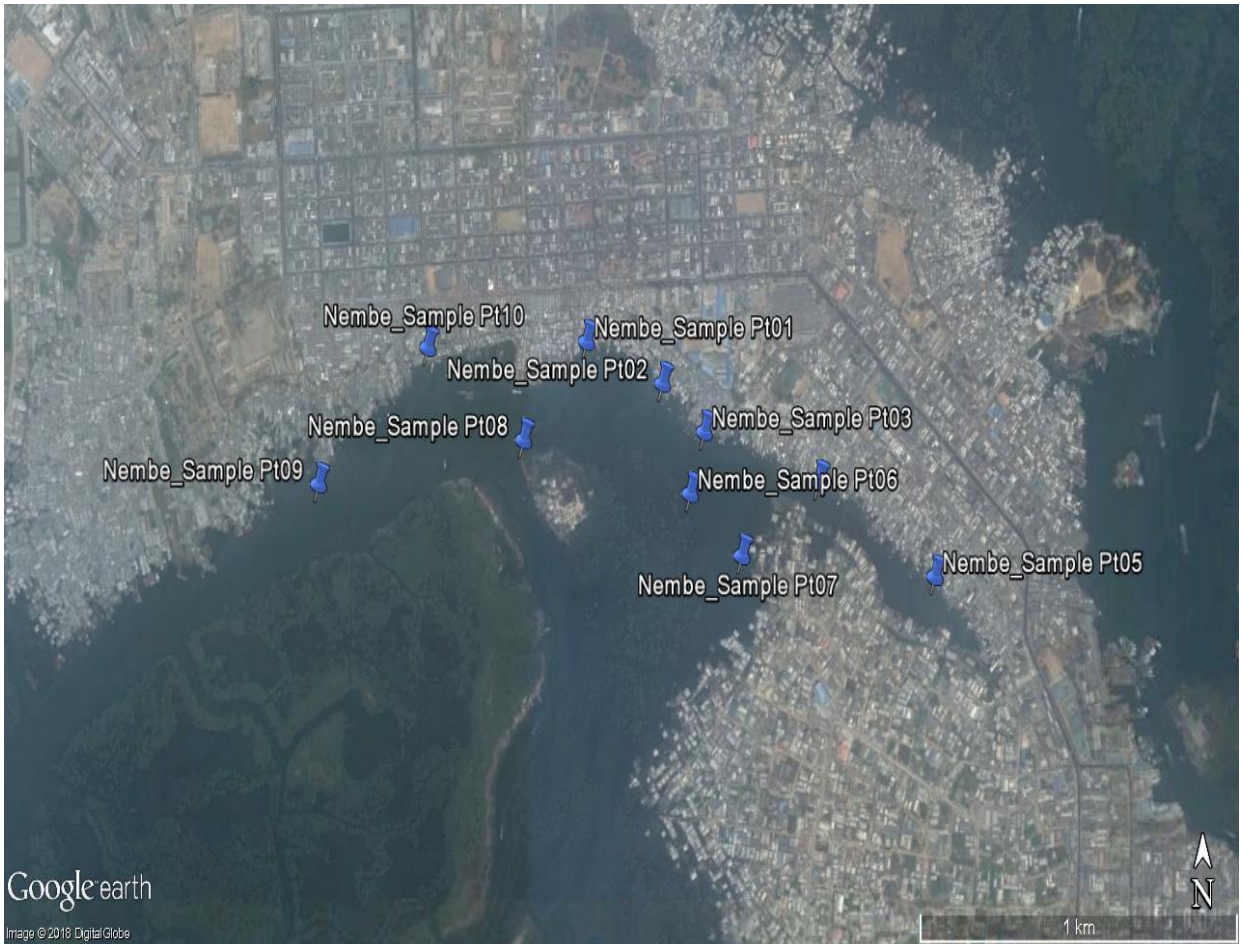


Plate 3.2: Geoeye satellite image (2016) showing the Nembe sample points
Source: DigitalGlobe (2018)

Plate 3.3: Geoeye satellite image (2016) showing the Onne sample points
Source: DigitalGlobe (2018)

3.2 Sample Collection, Preparation and Extraction

Ten random samples of marine sediment and water were collected from the ten (10) designated points of the three sampling sites. The samplings were done once in each of the the three sampling sites in September, 2015. The samples were mixed together to obtain a total of six composite /representative sediment and water sampleswhich were used for the analysis. The surface aerobic sediment samples were collected with a 95 % ethanol- sanitized plastic spatula at 5 cm depth into 95 % ethanol– sanitized clean, dry, leak – proof, wide mouthed plastic containers that is lined with aluminium foil. The water samples were collected at the air-water interface by hand dipping of the 95 % ethanol– sanitized clean, dry, leak – proof, cylindrical shaped 2 L plastic containers. The containers with lids slightly opened were rinsed with the samples thrice before aseptically collecting the samples. All the composite or representative sediment and water samplecontainers were labelled with sample type, date, time, and place of collection. They weer placed into a sterile polythene bagsin ice packed coolers to keep them under a temperature not more than 4 °Cand then transported to the Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria(Abu and Chikere, 2006; Gorleku *et al*., 2014; John *et al.*, 2012).The sediment–water suspensions (10:100 w/v) were prepared by shaking the sediments with sterile distilled water for 24 h at room temperature (25 ± 2 °C). The particle free extracts were obtained using membrane filtration technique by filtering the suspensions through glass fiber filters (d = 0.45 μm). Similarly, the marine water samples were prepared by filtering as previously described to separate the suspended and dissolved solids in the samplesprior to the physico -chemical, toxicological and microbiological analyses (Aruoja *et al*.,2011; Selivanovskay *et al.*, 2006).

3.3 Physico - chemical Analysis

The physico-chemical analysis carried out in this study include:

3.3.1 Total aromatic hydrocarbons (TAH) analysis

Byadopting the modified method described by AOAC (2012), 20 g of the homogenized sediment samples were mixed with 60 g of anhydrous sodium sulphate in Agate mortar to absorb moisture. The homogenates were placed into 500 ml beakers and extractions were carried out with 300 ml of n – hexane for 24 h. The crude extracts obtained were evaporated to dryness using a rotary vacuum evaporator at 40 $^{\circ}$C and the residues transferred with n – hexane onto 5 ml florisil column for clean up. Also, 10 ml of water samples were extracted with 200 ml of dichloromethane. The mixtures were separated using separating funnel and the dichloromethane layer was concentrated in rotary evaporator. One millilitre of acetonitrile was added into the concentrate and transferred into a vial ready for analysis. The florisil was heated in an oven at 130 $^{\circ}$C overnight (ca.15 h) and transferred to a 250 ml size beaker and placed in a desiccator. A 0.5 g anhydrous Na_2SO_4 was added to 1.0 g of activated flosiril (magnesium silicate) (60 – 100 nm mesh) on an 8 ml column plugged with glass wool. The packed column was filled with 5 ml n – hexane for conditioning. The stopcock was open to allow N – hexane run out until it just reaches top of sodium sulphate into a receiving vessel whilst tapping gently the top of the column till the florisil settled well in the column. The extract was transfered onto the column with disposable Pasteur pipette from an evaporating flask. Each evaporating flask was rinsed twice with 1 ml portions of n – hexane and added to the column. The eluate was collected into an evaporating flask and rotary evaporator to dryness. The dried eluate was dissolved in 1 ml n – hexane for TAH Chromatographic analysis. Gas flows to the columns, the inlets, the detectors, and the split ratios were adjusted. The flame ionization detectors were generally held at the high end of the oven temperature range to minimize the risk of analyte precipitation. All of these parameters were set to the correct values, but the instrument: scientific gas chromatographic system with flame

ionization detector equipped with an on – column, automatic injector, mass spectroscopy, HP 88 capillary column (100 m x 0.25 µm film thickness) (M530 Buck Scientific, CA USA) was double checked. The detector temperature of column A was held at 250 °C with injector temperature of 22 °C and integrator chart speed of 2 cm/minutes. The oven temperature was set at 180°C and the GC was allowed to warm up for 30 minutes. While it was warming up, the oven was programmed as follows: initial temperature was kept at 70 °C for 5 minutes; then heating resumed to 220 °C for 2 minutes and continued to 220 °C at a rate of 10 °C/minute. The rate of heating changed to a rate of 5 °C/minute to 280 °C. After the system was conditioned, 1 µl of each sample was injected onto column A using proper injection technique into the inlet, in the split mode of ratio 39.6:1 of carrier gas to the sample in the GC at a temperature of 250 °C, pressure of 231.2 kPa and flow rate of 173 ml/minutes. When the instrument was ready, the ‘’NOT READY’’ light turned off, and the run was started.

3.3.2 Total heavy metal analysis

Heavy metal analysis was conducted according to the method of APHA(2012). Two grams of sediment samples were weighed into a crucible and put into a muffle furnance for ashing at a temperature of 450 °C for 2 h. The samples were removed from the furnance and allowed to cool. The dry ashes were emptied into 250 ml beaker and 20 ml of 20 % H_2SO_4 were added, heated in a water bath for 20 minutes, filtered and made up to 50 ml with distilled water and stored in a sample bottle for atomic absorption spectroscopy (AAS) macro and micro nutrient analysis. Also, the water samples are thoroughly mixed by shaking and 100 ml was transferred into glass beakers of 250 ml volumes, to which 5 ml of concentrated nitric acid were added and heated to boil till the volume was reduced to about 15 - 20 ml, by adding conconcentrated nitric acid in increments of 5 ml till all the residues were completely dissolved. The mixtures were cooled, transferred to sample bottles and made up to 100 ml

using metal free distilled water. The samples were aspirated into the oxidising air-acetylene flame. When the aqueous sample were aspirated, the sensitivity for 1 % absorption was observed. A series of standard metal solutions of iron (Fe),cobalt (Co), copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), zinc (Zn), nickel (Ni),Mercury (Hg) andarsenic (As) in the optimum concentration ranges were prepared depending on whether the concentration of the samples is low or high. The reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/litre. A caliberation blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations. Heavy metal analysis was conducted using atomic absorption spectrophometer (FS240AA- Agilent, USA).

3.3.3 General parameter analysis

3.3.3.1 Conductivity determination

Byadopting the method described by AOAC (2012) as modified in this study, 10 g of 2 mm sieved dried sediment were weighed into an extraction cup and 10 ml of distilled water was added and the resulting mixtures were agitated on a mechanical shaker. Similar procedure was adopted for the water samples using 10 ml filtered sample volume. The conductivities were determined using a conductivity meter (DSS – 11A, China).

3.3.3.2 pH determination

The pH of the samples was determined usinga bench pH meter (PHS - 3CU, China) according to the method described by AOAC (2012) as modified in this study. The meter was first standardized against standard buffer solutions. The electrode of the meter was then

washed with distilled water and then immersed in the sample contained in a beaker. The pH of the sample was then read on the pH meter scale and noted.

3.3.3.3 Determination of percentage silt, clay and sand (Particle size analysis)

Byadopting the method described by AOAC (2012), 30 g of the sediment samples were weighed into 250 ml beakers and filled with distilled water up to 200 ml mark. The samples were washed four times with distilled water. Twenty millilitres of the solution of the freshly prepared 25 % sodium hexametaphosphate was added to 200 ml of distilled water-sample solution and was allowed to stand for 16 h. The suspension was transfered into 0.2 mm sieve. The samples on the sieve is the sand while the sample that pass through is the silt both of which were dried to a constant weight and the percentage of the particle sizes are given by:

$$\% \text{ Sand} = \frac{\text{Residue of sand}}{\text{Sample}} \times 100$$

$$\% \text{ Silt} = \frac{\text{Residue of silt}}{\text{Sample}} \times 100$$

$$\% \text{ Clay} = 100 - (\% \text{ Silt} + \% \text{ Sand})$$

3.3.3.4 Moisture content determination

The moisture content of the samples was determined according to the method described by AOAC (2012), as modified in this study. Approximately 2 g of the sediment samples were weighed into three Petri dishes that were washed and dried in the oven (DHG- 9053AA, Life Assurance Scientific, UK). The weight of the Petri dishes and samples were noted before drying. The Petri dishes and samples were transferred into the oven and heated at 105 $^{\circ}$C for 3 h, cooled in desiccators and the weight were noted. The drying procedure were continued until constant weights were obtained. The calculation of moisture content was given by:

% Moisture content = $\frac{100}{1}$

Where W1 = weight of Petri dish and sample before drying

W2 = weight of Petridish and sample after drying

3.3.3.5 Soil porosity and bulk density determination

According tothe method described by APHA (2012), and as modified in this study, particle density (as well as bulk density) of a sand was easily approximated in the laboratory by the following procedure. Exactly 50 g of dry sands were weighed and transferred quantitatively using funnel to a 100 ml graduated cylinder. The cylinder was tapped 4 times to settle the sands. The volume was read and recorded on data sheet and sedimented soil samples were oven - dried at 105 $^{\circ}$C for 2 h. The bulk density was calculated as follows:

Bulk density = oven - dry soil weight / volume of soil solids and pores

The sand particles were transferred to a container and saved. Fifty millilitres of water was added to the 100 ml graduated cylinder. The exact water volume was recorded (assume the density of water is 1 g c/m^3). Fifty grams of sandswere weighed and transferredinto the cylinder. It was stirred to remove the trapped air. The particle density was calculated by dividing the weight of the sand (50 g) by the volume of the sand particles as follows:

Particle density = oven-dry soil weight / volume of soil solids

Also, the pore space (% porosity) was calculated as follows:

Porosity = 1 – (particle density/bulk density)

3.3.3.6 Nitrogen determination

The nitrogen content of the samples was determined according to the method described by AOAC (2012). Exactly 1 g of sediment or 1 ml water samples were weighed or pipetted into

a 100 ml Kjeldhal flask gently and then the flasks were closed and shaken. Then, 1 g of the Kjeldhal catalyst was added. The mixture was heated cautiously in a digestion rack under fire until clear solutions appeared. The clear solutions were then allowed to stand and cool for 30 minutes. After cooling, 100 ml of distilled water was added to Kjeldhal flask to avoid caking and then transferred to the Kjeldhal digestion apparatus. A 500 ml receiver flask containing 5 ml of boric acid indicator was placed under a condenser of the distillation apparatus so that the tap was 20 cm inside the solution. Then, 10 ml of 40 % sodium hydroxide was added to the digested samples in the apparatus and distillation commenced immediately until distillation reaches the 35 ml mark of the receiver flask, after which it was titrated to pink colour using 0.01 N hydrochloric acid. The % nitrogen content was calculated using:

% Nitrogen = Titre value x 0.01 x atomic mass of nitrogen x 4

Where Titre value = Volume of 0.05 N boric acid and 0.01 = Normality of boric acid.

3.3.3.7 Phosphorus determination

The phosphorus content of the samples was determined using the method described by AOAC (2012). Exactly 100 ml of the homogenized and filtered samples were pipetted into a conical flask. The same volume of distilled water (serving as control) was also pipetted into another conical flask. One millilitre of 18 M H_2S0_4 and 0.89 g of ammonium persulphate were added to both conical flasks and gently boiled for 1 ½ h, making up the volume to 100 ml with distilled water. They were then cooled and one drop of phenolpthelein indicator was added and after neutralized to a faint pink colour with the 2 M Na0H solution. The pink colour was discharged by drop - wise addition of 2 M HCl, and the solutions were made up to 100 ml with distilled water. For the colorimetric analysis, 20 ml of the samples were pipetted into test tubes and 10 ml of the combined reagents added, shaken and left to stand for 10 minutes before reading the absorbance at 690 nm on a spectrophotometer using 20 ml of

distilled water plus 1 ml of the reagent as reference. The standard phosphate solution was prepared by weighing exactly 219.5 mg of dried potassium hydrogen phosphate, dissolved in distilled water andmade up to 1000 ml in the flask, where 1 ml = 50.0 µg of phosphate. 10 ml of the stock solution was made up to 1000 ml to give 1 ml = 0.05 mg. Standards of strength ranging from 0 (blank) to 0.05 mg/l at intervals of 0.01 mg was prepared by diluting the stock with distilled water. Phosphorus content was determined using the equation below:

$$\text{Concentration of sample} = \frac{\text{Absorbance of sample}}{} \times \text{Concentration of standard}$$

3.3.3.8 Potassium and calcium determination

Thepotassium and calciumcontent of the samples were determined according to the method described by AOAC (2012). Exactly 2 g of 2 mm sieved dried sediment samples were weighed into an extraction cup and 20 ml of ammonium acetate added at a pH of 7 and was agitated using a mechanical shaker for about 20 minutes and filtered using a 9 mm filter papers. The filtrates obtained were read using atomic absorption spectrophotometer (FS240AA- Agilent, USA).

3.3.3.9 Soil saturation determination

Byadopting the method described by AOAC (2012), cores were placed into sediment samples, trimmed flush with the metal cylinder at both ends, into a cylindrical sample holders and clamped tightly. The outside area of contact between the soil cores and the sample holders was sealed. The sample holders were placed into containers filled with water to a depth just below the top of the samples. The soil cores were allowed to soak for at least 16 h or until saturated. Then the time taken the water to drop completely was measured and soil saturation determined.

3.3.3.10 Total organic carbon determination

Thetotal organic carboncontent of the samples was determined using the method described by AOAC (2012), by determining **t**he moisture contents of the air – dried soils which have been grounded to pass a 0.42 mm sieve. Soils that could contain between 10 g and 20 mg of carbon were accurately weighed into a dry tared 20 ml conical flask (between 0.5 g and 1 g for 1 g for top soil and 2 g and 4 g for subsoil). Ten millilitres of 0.1 N $K_2Cr_20_7$ was added and swirled the flasks gently to disperse the soil in the solutions. Twenty millilitres concentration of H_2SO_4 was added thereby directing the stream into the suspensions. Immediately, the flask was swirled until the soils and the reagent were mixed. A 200 $^{\circ}$C thermometer was inserted and heated while the flasks were swirled with the contents on a hot plate or over a gas burner and guaze until the temperature reaches 139 $^{\circ}$C.The flasks were set aside to cool slowly on an asbestors sheet in a fume cupboard. Two blanks (without soil) were run in the same way to standardized $FeSO_4$ solution.When cool (20 – 30 minutes), the mixtures were diluted to 200 ml with deionised water, and proceed with the $FeSO_4$ titration using either the Ferroin indicator or potentiometrically with an expending scale pH/MV meter or auto titrator. For the Ferroin titration, 4 drops of Ferroin indicator were added and titrated with 0.4 N $FeSO_4$. As the end points was approached, the solutions took on a greenish colour and then changed to a dark green. At this point, the $FeSO_4$ was added drop- by- drop drop until the colour changed from blue – green to reddish – grey.

3.3.3.11 Soil texture (sediment type) determination

The soil texture (sediment type) of the three sampled locations were determined using soil texture triangle that gives a description of the various combinations of sand, silt and clay according to the method described by AOAC (2012). A coarse-textured or sandy soil is one

comprised primarily of medium to coarse size sand particles. A fine-textured or clayey soil is one dominated by tiny clay particles. Due to the strong physical properties of clay, a soil with only 20 % clay particles behaves as sticky, gummy clayey soil. The term loam refers to a soil with a combination of sand, silt, and clay sized particles. For example, a soil with 30 % clay, 50 % sand, and 20 % silt is called a sandy clay loam.

3.3.3.12 Total dissolved solids (TDS) determination

The total dissolved solids of the water samples were determined according to the method of APHA(2012). Firstly, the fiber filter discs were prepared by placing it, wrinkled side up, in the filtration apparatus. Vacuum was applied and the disc washed with three successive 20 ml washings of distilled water. Continuous suctions were then applied to remove all traces of water. Clean evaporating dishes were heated to 180 ± 2 ^{o}C in oven (DHG- 9053AA, Life Assurance Scientific, UK) for 1 h, cooled and stored in a desiccator until needed. They were weighed immediately before use. A sample volume was chosen to yield between 2.5 and 200 mg dried residues. Then, 50 ml of well mixed samples were filtered through the glass-fibre filters; washed with three successive 10 ml volumes of distilled water, allowing complete draining between washings. Suctions were continually applied for about 3 minutes after filtration is complete. The filtrates were transferred to a weighed evaporating dishes and evaporated to dryness on a steam bath (Techmel M3021, India). The evaporating dishes were finally dried for at least 1h in oven at 180 ± 2 ^{o}C, cooled in a desiccator to balance temperature and weighed. Total dissolved solid values were determined using the equation given below:

$$\text{TDS} = \frac{(A-B)^3 \text{ mg/l}}{\text{Sample volume in ml}}$$

Where A = weight of dish + solids (mg) and B = weight of dish before use (mg)

3.3.3.13 Total solids (TS) determination

Following the recommendation of the method described by APHA(2012), 100 ml of the water samples (50 ml) were measured into a pre-weighed dishes and evaporated to dryness at 103 ^{o}C on a steam bath. The evaporated samples were dried in an oven (DHG- 9053AA, Life Assurance Scientific, UK) for 1 h at 103 - 105 ^{o}C, cooled in a desiccator and their constant weights were recorded. Total solids were calculated as follow:

Total solids = Total Suspended Solids + Total Dissolved Solids

3.3.3.14 Total suspended solids (TSS) determination

Following the recommendation of the method described by APHA(2012), total suspended solids were determined by subtracting the result of total dissolved solids from total solid as follows:

Total solids (TS) – Total dissolved solids (TDS) = Total Suspended solids (TSS)

3.3.3.15 Chemical oxygen demand (COD) determination

The chemical oxygen demandof the water samples was determined according to the method of APHA(2012). Exactly 10.0 ml of the water samples were introduced into 100 ml round-bottom flask containing 2 ml potassium dichromate, 2.5 mlmercuric sulphate solution, 15 ml concentrated sulphuric acid containing silver sulphate and an anti – bumping rod. The mixtures were heated gently, but steady boiling over an electric hot plate under a reflux condenser. After exactly 45 minutes boiling, they were allowed to cool briefly, washed with 20 mlof distilled water through the condenser into the flasks and cooled completely in cold water. Two drops of Ferroin solution were added and titrated against excess potassium

dichromate with ammonium iron (II) sulphate until the colour changed from bluish- green to reddish brown. A blank with 10.0 ml distilled water under exactly the same conditions.

3.3.3.16 Dissolved oxygen (DO) in water determination

Following the recommendation of the method described by APHA(2012), the stopper from the water sample containers were carefully removed and added in turn 1 ml manganous sulphate solution followed by 1 ml alkaline – iodide –azide solution. The tips of the pipettes should be weeded below the surface of the liquidwhen introducing various reagents into the full container of samples. Thestoppers were carefully replaced after each addition so as to avoid inclusion of air bubbles. The contents were mixed thoroughly by inversion and rotation until a clear supernatant water was obtained. One millilitre of concentrated sulphuric acid was added with the tip of the pipette below the level of solutions and again replaced the stoppers. The solution was mixed well by rotation until the precipitation has completely dissolved. 100 ml of the solutions were pipetted into 250 ml conical flasks and immediately titrated against standard sodium thiosulphate (0.0125 mol/dm^3) using freshly prepared starch solution as the indicator which was added when the solution became pale yellow. The titration was carried out in triplicate.

3.3.3.17 Biological oxygen demand (BOD_5) determination

Following the recommendation of the method described by APHA(2012), the general equation for the determination of a BOD_5value is given below:

BOD_5 (mg/l) ÷ $\frac{D_1 - D_5}{0.05}$ = initial dissolved oxygen (DO) of the sample; D_5 = final dissolved oxygen (DO) of the sample after 5 days; and P = decimal volumetric fraction of sample used (0.05).

3.4 Acute Toxicity Testing

3.4.1 Analytical chemicals and reagents

The aromatic compounds namely xylene, anthracene and pyrene used in this study were selected as revealed in the chemical characteristics of both sediment and watersamples and are representatives of simple, lower and higher molecular weight aromatic hydrocarbons.

Xylene of analytical grade was purchased from MERCK (Pty) Limited, South Africa (CAS NO: 1330 - 20 -7, C_8H_{10}, MW: 106.17 g/mol: MP: -34 °C, BP:136 °C, VP: 8.29 at 25 °C). The test chemical is greater than or equal to 98.5 % pure (HPLC). Different concentrations (400 mg/kg, 1000 mg/kg and 2000 mg/kg) of xylene were prepared using olive oil as diluent for the animal study.

Anthracene of analytical grade was purchased from MERCK (Pty) Limited, South Africa (CAS: 129 - 00 - 0, $C_{14}H_{10}$, MW: 178.23 g/mol, MP: 213 - 216 ^{0}C, BP: 342 ^{0}C). The test chemical is greater than or equal to 96 % pure (HPLC). Different concentrations (100 mg/kg, 500 mg/kg and 1000 mg/kg) of anthracene were prepared using olive oil as diluent for the animal study

Pyrene of analytical grade was purchased from Sigma Aldrich, United Kingdom (CAS: 129 – 00 - 0, $C_{16}H_{10}$, MW: 202.25 g/mol, MP: 145-148 °C (lit.), BP: 404 °C). The test chemical is greater than or equal to 98 % pure (HPLC). Different concentrations (50 mg/kg, 120 mg/kg and 250 mg/kg) of pyrene were prepared using olive oil as diluent for the animal study.

Benzo (a) pyrene of analytical grade was purchased from Sigma Aldrich, China (CAS: 50-32-8, C_2OH_{12}, MW: 252.31 g/mol, MP: 177-180 $^{\circ}$C (lit.), BP: 495 $^{\circ}$C (lit.)). The test chemical is greater than or equal to 96 % pure (HPLC). Different concentrations (20 mg/kg, 40 mg/kg and 100 mg/kg) of benzo (a) pyrene were prepared using olive oil as diluent for the animal study and use as standard PAH.

Toxi-Chromotest^{TM} kit was purchased from Environmental Biodection Products Inc. Canada, while Marine ALGALTOXKIT and Artoxkit M were purchased from MicroBiotests Inc. Belgium and used for toxicity testing study.

The acute toxicity testing of the sediment and water samples of three sampled locations as well as the representative aromatic hydrocarbons mentioned above was carried out based ona battery of tests involving representatives of all organisms and trophic levels in the ecosystem to detect the signal of the impact of all the chemicals present in the marine ecosystem. The plant (producer) bioassay via marine algal toxicity test using *Phaeodactylum tricornutum* (microalgae) and phototoxicity test using *Sinapsis alba*(mustard seeds); the invertebrates (consumer) bioassay via *Artemia* toxicity test using*Artemia franciscana*(brine shrimps)and bacteria (decomposer) bioassay via Toxi - Chromotest using genetically engineered *E. coli* were adopted in this study.

3.4.2 Marine algal toxicity test

The marine toxicity test for microalgae was carried out with marine algaltoxkit according to the standard method of EBPI (2016) as follows:

3.4.2.1 Preparation of algal culturing medium (synthetic seawater)

Approximately 1,500 ml of deionized water was poured into 2 L volumetric flask and thecontents of vial number 1 (NaCl) was poured into the flask and shaken until all the salt was dissolved. Similarly, the contents of the vials with concentrated salt solutions labelled number 2 (KCl), number 3 ($CaCl_2$), number 4 ($MgCl_2$), number 5 ($MgSO_4$), number 6 ($NaHCO_3$) and number 7 (H_3BO_3) were uncapped and poured into the 2 L volumetric flask.

3.4.2.2 Addition of nutrient stock solutions

Thirty millilitres (2 x 15 ml) of stock solution A, 1 ml of stock solution B and 2 ml of stock solution C were added to the 2 L volumetric flask. Five hundred millilitres of deionized water was added up to the 2, 000 ml mark and shaken to homogenize the medium.

3.4.2.3 Preculturing of the alga

One of the two tubes containing the microalga inoculum was taken, handshaken vigorously and the content was poured into one of the pre-culturing cells.The (same) tube was rinsed twicewith 7.5 ml algal culturing medium and the content was transferred into the pre-culturing cell to ensure the total transfer of the microalga inoculum.The pre - culturing cell was closed with the lid and incubated for 3 daysin an incubator (Kottermann D3165, West Germany) at 20 ± 2 °C under aconstant uniform illumination supplied by cool white fluorescent lamps.

3.4.2.4 Preparation of concentrated algal inoculum

The long cell with the label "Calibration long cell" was filled with 25 ml algal culturing medium and closedwith the lid. The cell was placed in the spectrophotometer (BA – 88A MINDRAY, Germany), and the instrument was zero-caliberated. The pre-culturing cell was taken and shaken to homogenize the algal suspension after the 3 days of incubation. The cell was placed in the spectrophotometer and the optical density (OD1) was read at 670 nm after 10 seconds. The number of alga (N1) corresponding with OD1 was interpolated from the optical density/algal number (OD/N) standard curve. The N1/N2 ratio was calculated inorder to determine the dilution factor needed to reach an optical density equal to OD2, corresponding to an algal density of 1.10^6 cells/ml with N2 equal to 1.10^6 algal cells/ml. The algal suspension from the pre - culturing cell was transferred into a 100 ml sterile flask and the volume of algal culturing medium needed to make up a 1.10^6algal cells/ml suspension was added and the flask shaken thoroughly to distribute the algae evenly. The pre-culturing cell was rinsed and 25 ml of the 1.10^6 algal cells/ml was transferred into this cell, the lid on the cell was placed, shaken gently and the OD was read after 10 seconds. The OD value was finally checked on the OD/N standard curve to knowif the OD corresponds with the desired OD2 value (1.10^6 algal cells/ml).

3.4.2.5 Preparation of the toxicant dilution series

Eight (8) calibrated flasks of 100 ml contents were labelled as 'Stock 1' and 'Stock 2' while the others C0, C1 to C5. Hundred milligramsof potassium dichromate were weighed on an analytical balance and transferred into the 'Stock 1' flask. Algal culturing medium was added to the 100 ml mark and shaken to dissolve the chemical and obtained a 1 g/l (Stock 1) concentration. Ten millilitres from 'Stock 1' was transferred into 'Stock 2' flask and filled to the 100 ml mark with algal culturing medium. The contents were shaken to homogenize and obtained a 100 mg/l (Stock 2) toxicant concentration. The following volumes of toxicant

solution was transferred from 'Stock 2' into the following flasks: - 18 ml to flask C1 (18 mg/ml), 5.6 ml to flask C2 (5.6 mg/ml), 3.2 ml to flask C3 (3.2 mg/ml, 1.8 ml to flask C4 (1.8 mg/ml) and 1.0 ml to flask C5 (1.0 mg/ml).The algal culturing medium was added up to the 100 ml mark in the C0, C1, C2, C3, C4 and C5 flasks. Zero point nine millilitre of the 1.10^6 cells/ml of algal stock in the tube was added to each flask (except the stock flasks), in order to obtain an initial concentration of 1.10^4 algal cells/ml in each toxicant concentration. The flasks were closed and shaken thoroughly to distribute the algal suspension evenly.

3.4.2.6 Reference test

A quality control test was carried out with the reference chemical potassium dichromate ($K_2Cr_2O_7$) and all the steps inpreparation of the toxicant dilution series were repeated in like manner.

3.4.2.7 Transfer of the algal - toxicant dilutions into the test vials

The holding trays were taken, the rubber bands and the plastic strips were removed, and the long cells in sets of 3 (a, b, c) for each concentration (from CO to C5) were marked. All the cells were opened by lifting up one end of the lids. After thorough shaking, 25 ml of the algal -toxicant dilutions from each flask were transferred into the corresponding 3 long cells. All cells were opened and shaken. The OD value of each long cell was determined in the spectrophotometer (BA – 88A MINDRAY, Germany)and expressed as 0 h (T0) OD values. The T0 data were recorded on the results sheet.

3.4.2.8 Incubation of the test vials

All the long cells were put back into their holding trays and all the lids on the same side were lifted up slightly and the plastic strips were slided over the open part of the long cells, taking

care to leave an opening near the middle of the long cells for gas exchange. The long cells were placed in the holding tray in a random way (that is not in the sequence of C0 to C5, and not all three parallels next to each other), in order to compensate for possible small "site to site" differences during incubation. The holding trays were incubated for 3 daysin an incubator (Kottermann D3165, West Germany), at 20 ± 2 °C under aconstant uniform illumination supplied by cool white fluorescent lamps.

3.4.2.9 Scoring of the results

The inhibition of the algal growth relative to the control was determined by daily measurement of the OD of the algal suspensions in the long cells during the 3 days of the test, i.e. after 24 h, 48 h and 72 h exposure to the toxicant. The long cells were put back in the holding trays in a random way after the daily measurement. The daily results for each long cell were recorded on the results sheets.

3.4.2.10 Data treatment and expression of results

The mean daily OD values for the 3 replicate long cells of each toxicant dilution were calculated. The algal growth inhibition from these data was calculated by integrating the mean values from t0 to t72 h (termed as the "area under the curve") for each concentration tested, including the control. The growth curves for each test concentration and control as a graph of the logarithm of the mean cell density against time was plotted. The average specific growth rate (μ) for each test culture was calculated using the equation:

$$\mu = \frac{\ln NL - \ln No}{tL - t0}$$

where: *t0* is thetime of test start; *tL* is the time of test termination or th time of the last measurement within the exponential growth period in the control; *No* is the nominal initial

cell density; *NL* is the measured cell density at time *tL*. Alternatively, it was also calculated from the slope of the regression line.

The percentage growth inhibition for each test concentrations was calculated using the equation:

$$I\mu i = \frac{\overline{\mu c} - \overline{\mu i}}{\mu c} \times 100$$

Where: $I\mu i$ is the percentage inhibition (growth rate) for test concentration *I*; $\overline{\mu i}$ is the mean growth rate for test concentration *i*;$\overline{\mu c}$ is the mean growthrate for control *c*.The effective concentration (E_rC_x)can be determined by tabulating and plotting the normalized inhibition ($I\mu i$) against the test concentration on the logarithmic scale. A linear model was suitably fitted to the experimental data points by probit regressional analysis and the median effective concentration (E_rC_{50}) was determined by the equations:

Y= Slope X + Intercept Y and Antilog EC_{50}.

3.4.3 Phytotoxicity test

The germination toxicity experiment was carried out using the method of Emami *et al.* (2014)and EBPI (2016) as modified in this study. Onecubic decimetre of non-polluted garden soil was oven-dried by placing it in electric oven (DHG- 9053AA, Life Assurance Scientific, UK) for 1 h. The oven - dried soil was sieved through a sieve with a 2 mm mesh to eliminate all coarse materials. A 100 ml beaker was filled to the 90 ml mark with the soil and 30 ml distilled water was slowly added and mixed thoroughly with the soil till all the soil is wet. The soil/water mixture was allowed to reach equilibrium leading to a water-saturated soil phase with a water layer on top. Thirty grams of the corresponding hydrated soil sample were evenly introduced into plastic Petri dishes of 90 x 15 mm, and the surface flatten to obtain a

soil layer of uniform depth. White filter papers (Whatman No. 1) were placed on the surface of the hydrated uniform depth soil and allowed to be completely wet. Twenty millilitresof chemicals (xylene, anthracene, and pyrene) and sediment (Abonema, Nembe, and Onne) solutions (250 mg/kg for solid test sample or mg/l for liquid test sample) were pipetted and slowly spread over the entire surface of the white filter paper while boric acid (250 mg/kg) and distilled water were used as positive and negative controls.Ten seeds of the selected test plant *Sinapis alba* (mustard seeds) were placed on top of the white filter paper, in one row and at equal distance of each other. The seeds were being placed near the top of the filter paper, at about 1 cm of the middle ridge of the test plate. The plates were covered and appropriately labelled with respective samples in three sets.The test plates (samples and controls) inoculated with the test plant seeds were carefully placed in the incubator (Kottermann D3165, West Germany) and incubated at 25 ± 2 °C for 3 days in darkness.After the 3 days of incubation, the number of germinated seeds and the length of each roots were counted and measured and the mean triplicate values were used to determine the germination indexdefined as:

GI = 100 × (Gs/Gc) × (Ls/Lc) where Gs and Gc are mean germinated seeds in the sample and control; and Ls and Lc are mean root elongation in the sample and control respectively. The mean values (± S.D.) were compared using ordinary one-way ANOVA and Dunnett's multiple comparison test to determine the if there was difference in phototoxic effect among the treatment group of aromatic hydrocarbons, sediment samples and the controls.

3.4.4*Artemia* toxicity test

Artemia toxicity screening test was carried out with Artoxkit M according to the standard method of EBPI (2016) as follows:

3.4.4.1Preparation of the standard seawater

Approximately 800 ml of deionized water was poured into 1 L volumetric flask and the contents of vial number 1 (NaCl) was poured into the flask and shaken until all the salt was dissolved. Similarly, the contents of the vials with concentrated salt solutions labelled number 2 (KCl), number 3 ($CaCl_2$), number 4 ($MgCl_2$), number 5 ($MgSO_4$), number 6 ($NaHCO_3$) and number 7 (H_3BO_3) were uncapped and poured into the 2 L volumetric flask. Two hundred millilitres of deionized water was added up to the 1, 000 ml mark and shaken to homogenize the medium.

3.4.4.2Hatching of the *Artemia* cysts

Artemia cyst hatching was initiated 30 h before the start of the toxicity test. The content of one vial with brine shrimp cysts was emptied into one of the small Petri dishes making sure that most of the cysts are transferred. Ten millilitres of standard seawater was added into the Petri dish and swirled gently to distribute the cysts evenly. The hatching Petri dish was covered, was incubated for 30 hin an incubator (Kottermann D3165, West Germany) at 20 ± 2 °C under aconstant uniform illumination supplied by cool white fluorescent lamps. Hatching will start after about 18 - 20 h and after 30 h, most of the larvae would have moulted into the instar II-III stage.

3.4.4.3Preparation of the toxicant dilution series

One 100 ml volumetric calibrated flaskwas labelled as ‘Stock’while six 10 ml test tubes were labelled C0, C1 to C5. One hundred milligramsof potassium dichromate were weighed on an analytical balance and transferred into a 100 ml volumetric flask. The standard seawater was added to the 100 ml mark and shaken to dissolve the chemical and obtain a stock solution of 1 g/l potassium dichromate. One millilitre from the stock solution was transferred into C1

tube and filled to the 10 ml mark with standard seawater. The content was shaken to homogenize and obtained a 100 mg/l toxicant concentration. The following volumes of toxicant solution were transferred from C1 tube into the following tubes: 0.56 ml to tube C2 (56 mg/ml), 0.32 ml to tube C3 (32 mg/ml), 0.18 ml to tube C4 (18 mg/ml) and 0.10 ml to tube C5 (10 mg/ml).

3.4.4.4 Reference test

A quality control test was carried out with the reference chemical potassium dichromate ($K_2Cr_2O_7$) and all the steps inpreparation of the toxicant dilution series were repeated in like manner.

3.4.4.5 Filling of the test plate

3.4.4.5.1 Controls

One millilitre of sterile dilution water was added to each well of column 1 (wells A1, B1, C1 and D1).

3.4.4.5.2 Toxicant dilutions

One millilitreof test tube labelled 5 was transferred to each well of column 2 (wells A2, B2, C2, D2) after all test tubes were shaken thoroughly for all the test samples as well as positive control. The same procedures were repeated with test tubes labelled 4,3,2 and 1 inorder to fill the wells of columns3,4,5 and 6 respectively.

3.4.4.5.3 Transfer of the larvae to the test wells

The hatching Petri dish was taken out of the incubator and was allowed for approximately five minutes to allow the nauplii to congregate. The Petri dish was placed under the microscopic stage and approximately 50 instars II - III larvae weretransferred with a

micropipette from thePetridish to each well in row D (rinsing wells) of the multiwell plate in the following sequence: D1 (control), D2, D3, D4, D5 and D6 (increasing concentrations of toxicant) using a dissection microscope (WF10X/23 Chongqing Optec, China)at magnification 10 – 12X.The multiwell plate was placed on the stage of the dissection microscope and 10 larvae were transferred from the rinsing well of column D1 to the three test wells of column 1 (C1, B1 and A1).The transfer was repeated for columns 2, 3, 4, 5 and 6 in all the test samples and positive control respectively.

3.4.4.6 Incubation of the test plates

Theparafilm strip was placed on top of the multiwell plates with the covers placed tightly. The multiwell plates were placed in the incubator (Kottermann D3165, West Germany) at 27 ± 2 °C in darkness for 24 hunder aconstant uniform illumination supplied by cool white fluorescent lamps.

3.4.4.7 Scoring of the results

The multiwell plates were taken out of the incubator and placed under the dissection microcope(WF10X/23 Chongqing Optec, China). The wells of rows A, B and C were checked and the number dead and living larvae were recorded on the result sheet. The number of dead larvae for each concentration were totaled and the percentage mortality was calculated.

3.4.4.8 Estimation of the LC_{50}

By adopting the method of Khoshnood *et al.* (2017), a linear model was suitably fitted to the experimental data points obtained in the test and reference control by Probit regressional

analysis and the 24 hmedian lethal concentration (LC_{50}) was determined by the equations: Y= Slope X + Intercept Y and Antilog $LC_{50.}$

3.4.5Toxi-chromo test

The Toxi-chromotest was carried out according to the standard method of EBPI (2016) toxi-chromotest procedure version 4.0 as follows:

3.4.5.1 Plate preparation

One hundred microlitres of diluent from Bottle G was dispensed to all other wells except row A containing undiluted sample and standard. Two hundred microlitresof standard toxicant (Bottle D) and 200 µl of the 100 % concentration of samples were dispensed to the appropriate wells of Row A. The required two-fold serial dilutions of each samples (marine wastewater and distilled water spiked with xylene, anthracene and pyrene) and the standard toxicant ($HgCl_2$) were prepared by transferring 100 µl from well A of each column into the next well (B) and continued by serially transferring 100 µl until Well G and more dilutions starting in row A of the next column and down the column again were done to end with 13 dilutions per sample. One hundred microlitresof the diluent and highest sample concentration were dispensed to the wells in row H of the columns containing that sample to create reagent blanks. To the blank row (H), 100 µl of reaction mixture (bottled A) was dispensed to all wells of row H. These wells are the reagent blanks and contain no bacteria.

3.4.5.2 Bacterial preparation and addition

The rehydration solution in bottle C was transferred to bacteria in Bottle B, mixed and left at 25 ± 2 °C for 15 minutes for complete rehydration. One millilitre of rehydrated bacteria from Bottle B was transferred to reaction mixture in Bottle A. One hundred microlitresof reaction mixture including bacteria (from Bottle A) was dispensed to all wells in the microplate except the reagent blank (row H). The plates were incubated in the incubator (Kottermann D3165, West Germany) at 37 °C for 90 minutes.At this time, the chromogen (Bottle F) was warmed by placing it in the incubator.

3.4.5.3 Chromogen addition and colour development

The plates were removed from the incubator and 100 µl of the warmed chromogenic substrate (bottle F) was dispensed to all wells of the plates. The plates were incubated in an incubator at 37 °C for30 minutesuntil a blue colour developed inthe zero concentration wells or negative control wells (column 2).

3.4.5.4Reading and analysis of results

The results were read quantitatively by measuring absorbance at 630 nm once sufficient blue colour development has occurred using a micro plate reader (MR – 96A MINDRAY, Germany). The absorbance values of the results were recorded and toxicity factor (TF), coeffiecient of variation (CV) and median effective concentration (EC_{50}) using GraphPad Prism 7.04 software for the individual samples were calculated and plotted.

Toxicity factor of sample was calculated by the equation:

$TF = 100 - 100\ [(A_{600}S - A_{600}BLavg)/ (A_{600}NCavg - A_{600}BLavg)]$

Where $A_{600}BLavg = (A_{600}BL1 + A_{600}BL2 + A_{600}BL3 + A_{600}BL4)/ 4$

$A_{600}NCavg = (A_{600}NC1 + A_{600}NC2 + A_{600}NC3 + A_{600}NC4 + A_{600}NC5 + A_{600}NC6 + A_{600}NC7)/ 7$

Coefficient of variation was also calculated by the equation:

CV = 100 x (SD/avg TF)

CV ≤ 25 % for replicates to be valid

The median effective concentration (EC_{50}) was calculated and determined by the non – linear equation: Y=Bottom + (Top-Bottom)/ (1+10^ ((LogEC_{50}-X) *HillSlope))

3.5 Sub – Chronic Health Effects

3.5.1 Laboratory animals

Adult Wistar albino mice (13.17 g - 30 g body weight, 12 weeks of age) were purchased at the Christian Farm beside General Hospital, Mgbakwu, Awka North L.G.A. Anambra State Nigeria and used in this study (78 mice). Mice were housed in aluminium wire cages, and maintained on a standard commercial diet. They had free access to water and food and were housed 6 mice/ aluminium wire cage and maintained in a controlled room at 25 ± 3 °C in a 12 h light: dark cycle, for one week (for acclimatization) before the start and throughout the experimental period (35 days). The mice where allowed *ad libitum* to grower feed of poultry (TOP VITAL FEEDS, Kano State, Nigeria) and water. The animals were handled in accordance with the principle of laboratory animal care (Issa and El-Sheriff, 2015; Morcos *et al*., 2015).

3.5.2 Establishment of animal models

The animal models were established as described by standard method of EU (2010). Briefly, twenty-four (24) Adult Wistar albino mice were used for preliminary experiments to determine suitable dosages for the aromatic hydrocarbons.

3.5.3 Sub-chronic animal studies

A total numberof 78 adult Wistar albino mice at 1:1 sex ratio were assigned to a control group and 4 treatment groups. The test chemicals (xylene, anthracene, pyrene and benzo (a) pyrene (standard)) were dissolved in olive oil and 0.1 ml was administered orally. After one week of acclimatization, the animals were weighed and randomly divided into five groups (6 mice in group 1 and 18 mice each in groups 2, 3, 4 and 5). Groups 2, 3, 4 and 5 were subdivided into 3 subgroups (6 mice per subgroup) according to the dose concentrations (400, 1000 and 2000 mg xylene/kg; 100, 500 and 1000 mg anthracene/kg; 50, 125 and 250 mg pyrene/kg and 20, 40 and 100 mg benzo (a) pyrene/kg). Group 1 was administered orally with 0.1 ml olive oil only and served as control while groups 2, 3, 4 and 5 were treated with xylene, anthracene, pyrene and benzo (a) pyrene and administered with similar doses daily for 35 days (Issa and El-Sheriff, 2015; Morcos *et al*., 2015; Wang and Xue, 2015).

3.5.4 Body and organ weight determination

After the experimental period (35 days) sub-chronic exposure of the hydrocarbons treated mice, the weights of the animals were determined and the blood samples were collected in a vaccum blood collection tube (Agary) by cardiovascular aspiration puncture using agarine sterile insulin syringe (Wuxi-Yoshou, China) containing anticoagulant (EDTA). Plasmacells were obtained by centrifugation of the blood at 300 rpm for 10 minutes. The clear supernatant was aspirated by means of Pasteur pipette and stored at -20 °C until use (Morcos *et al*., 2015). In the same vein, the tissues (stomachs, livers, kidneys and lungs) were removed intact by dissecting the mice, weighed and fixed in 10 % neutral buffered formalin (Issa and El-Sheriff, 2015; Okani *et al*., 2013; Wang and Xue, 2015).

3.5.5 Haematological analysis

By adopting the method of Morcos *et al*. (2015), the haematological parameters used in this study includes white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The blood haematological levels were analyzed at Comax International Laboratory Ogidi, Anambra State, Nigeria.

3.5.6 Biochemical analysis

By adopting the method of Morcos *et al*. (2015), the biochemical parameters observed in this study include total bilirubin (TB), direct bilirubin (DB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), creatinine (CRE), urea (UR), potassium (K), sodium (Na), chloride (Cl), and bicarbonate (HCO_3). The blood biochemistry levels were analyzed by Comax International Laboratory Ogidi, Anambra State, Nigeria.

3.5.7 Histopathological analysis

Partial embedding method was employed as described by Vainer *et al*. (2011). The tissues were sectioned into horizontal slices. Apex and basis were cut sagittally, and some of the remaining slices were embedded in quadrants. The paraffin-embedded sections were stained with haematoxylin and eosin (H and E stain) and slides were systematically examined by compound microscope (CX 23 Olympus, Japan) for histopathologically effects at Ifes Specialist Diagnostic Laboratory and Clinic, Awka, Anambra state, Nigeria.

3.6 Bacteriological Analyses

3.6.1 Preparation of media

The modified mineral basal medium was prepared and used for isolation hydrocarbon-utilizing bacteria and bioremediation study which contain the following composition: 4 g K_2HPO_4, 1.0 g$(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g $FeSO_4$, 0.1 g NaCl, 0.2 ml $CaCl_2$, 25 g Agar agar and brackish water (mixture of salt and fresh water) 30 ppt-1,000 ml at pH 7 ± 0.2. This was amended with 0.2 ml of acetone solution containing 0.1 % w/v of each hydrocarbon. Nutrient agar was prepared and used as the medium for isolation of heterotrophic bacteria according to the manufacturer's instruction (Esedafe *et al.*, 2015; John and Okpokwasili, 2012; Kafilzadeh *et al*., 2012). Seawater Nutrient Medium (SWNM: 5.0 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 500 ml aged seawater and 500 ml deionised water amended with 10 mg/l of each heavy metals.

3.6.2 Sterilization of materials

As stated in Willey*et al*. (2008), conical flasks (Pyrex), prepared media and other plastic materials were sterilized by autoclaving at 121 $^{\circ}$C for 15 minutes at a pressure of 15 psi. Glass wares such as pipettes, glass spreader, Petri dishes, measuring cylinder, and other glass materials were sterilized in the laboratory hot air oven at a temperature of 160 $^{\circ}$C for 1 h before use.

3.6.3 Dilution technique

A ten-fold serial dilution of the samples was carried out by adding 1 g or 1 ml respectively of sediment and water samples aseptically into test tubes containing 9 ml of 0.85 % of physiological saline solution labeled 10^{-1} to 10^{-10} dilution factors with the aid of a sterile pipette in a repeated manner. With another sterile pipette, 0.1 ml aliquots of the appropriate dilutions (dilutions that produce colony counts between 30 - 300 colonies) were spread plated on the surfaces of the solidified media in triplicates with the aid of a glass spreader. Precisely,

10^{-3} dilutions were spread plated. The spreader was sterilized after each successive spreading by dipping it in 70 % ethanol and then passing it through flame of a Bunsen burner (Bahig *et al*., 2008; Chikere *et al.*, 2009; Kafilzadeh *et al*., 2012).

3.6.4 Enrichment culturing technique

3.6.4.1 Enrichment, culturing and isolation of aromatic hydrocarbon - degrading bacterial strains

The hydrocarbon degraders were isolated from sediment and water samples of the three sampling sites using modified mineral basal agar (4 g K_2HPO_4, 1.0 g$(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g $FeSO_4$, 0.1 g NaCl, 0.2 ml $CaCl_2$, 15 g Agar agar and distilled water 1,000 ml at pH 7 ± 0.2) enriched with xylene, anthracene and pyrene as sole carbon and energy source. The medium was sterilized by autoclaving at 121 $^{\circ}$C and 15 psifor 15 minutes. Thereafter, 0.2 ml acetone solution containing 0.1 % w/v of the selected hydrocarbons (xylene, anthracene and pyrene) were aseptically pipetted and uniformly spreaded on the agar surface of the pre-dried Petri dish plates. The acetone was allowed to evaporate under sterile condition before inoculating with 0.1 ml of diluted sediment and water samples. The inoculated plates were sealed using adhesive tape and foil to prevent contamination and photolysis and later placed in black polythene bags, and then incubated in the dark at 28 ± 0.2 °Cfor 14 days. Also, Nutrient agar plates without hydrocarbons were inoculated and incubated at 28 ± 0.2 °C for 24 – 48 h(Chikere *et al.*, 2009; Esedafe *et al.*, 2015; John *et al*., 2012; John and Okpokwasili, 2012).

3.6.5 Total viable count technique

3.6.5.1 Enumeration of total heterotrophic bacteria

The total heterotrophic bacterial count was determined on nutrient agar using spread plate method. All the plates yielding 30 - 300 colonies were counted and the average number of colonies per plates were determined. The numbers of total heterotrophic bacteria were expressed at LogCFU/g and LogCFU/ml (Bahig *et al*., 2008; Chikere *et al.*, 2009; Esedafe *et al.*, 2015; Kafilzadeh *et al*., 2012; Isiodu *et al.*,2016).

3.6.5.2 Enumeration of total culturable hydrocarbon-utilizing bacteria

The total culturable hydrocarbon-utilizing bacteria were enumerated on the modified mineral basal agar using the spread plate method. All the plates yielding 30 - 300 colonies were counted and the average number of colonies per plates were determined. The numbers of total hydrocarbon utilizing bacteria were expressed at LogCFU/g and LogCFU/ml(Chikere *et al.*, 2009; Esedafe *et al.*, 2015; Kafilzadeh *et al*., 2012).

3.6.6 Purification and maintenance of cultures

Colonies that developed on hydrocarbon-coated plates were replicated onto fresh hydrocarbon-coated agar plates and incubated for 14 days. Isolates that grew on these plates were selected as xylene, anthracene and pyrene degraders and sub-cultured on Bijou bottles where they are preserved at 4°C in refrigerator (John *et al*., 2012).

3.6.7 Screening and selection test

In order to screen and select the best and strongest degrading strains, growth of the different organisms was tested by growing 5 ml of each desired isolates in large test tubes containing 25 ml of the modified mineral basal medium with 1 ml of xylene, anthracene and pyrene hydrocarbons which were dissolved in acetone and added to each tube after autoclaving.

Thereafter, the test tubes were incubated at room temperature (28 ± 2 °C) for five days. Bacteria that started growing fast with high turbidity in the vicinity of the medium containing aromatic compounds measured at 600 nm using a UV-VIS spectrophotometer (Astell, UV-Vis Grating, 752W) were selected as the candidate of xylene, anthracene and pyrene degrading bacteria. Cultures without increase in turbidity over initial optical density (OD) and uninoculated control were scored as no growth (-) while cultures with increased turbidity significantly greater than the control were scored as growth (+) (John *et al*., 2012; Kafilzadeh and Pour, 2012; Yuliani *et al.*, 2012).

3.6.8 Phenotypicalidentification of selected hydrocarbon - utilizing bacterial isolates

3.6.8.1 Morphological characteristics

3.6.8.1.1 Colonial morphology

After sub-culturing and incubation,culturing morphological properties such as shape, elevation, margin, optic, texture, colour, size and surface characteristics of the selected bacterial strains were observed and noted (Willey*et al*., 2008).

3.6.8.1.2 Microscopic morphology

3.6.8.1.2.1 Gram staining

This technique divides bacteria into Gram positive and Gram negative groups. A smear of the isolate was made on clean dry grease-free slide, using a sterile wire loop. The smear was air-dried and heat fixed by passing over flame quickly three times. It was then covered with 0.5 % crystal violet solution for 1 minute and rinsed with distilled water. The slide was flooded with 1 % Gram's iodine (which served as a mordant that fixes the dye inside the cell). The iodine was washed off after one minute and 95 % ethanol was used to decolorize the smear for 30 seconds. The smear was counter-stained with 0.1 % safranin dye solution for one

minute. It was then washed off and the slide air-dried, and observed under the microscope using oil immersion objective lens after placing a drop of oil immersion. Gram positive and negative reactions were indicated by purple and red colours respectively (Cheesbrough, 2006).

3.6.8.1.2.1 Spore staining

According to the method of HPA (2007), smears of the isolates were prepared and fixed on a slide. The underside was vapor heated and flooded with 5 % Malachite green solution. Heating would continue until visible water condensate forms under the slide with evaporation at the top. It was the washed using distilled water. Smears were counter stained with 0.5 % safranin solution for 10 seconds. Slides were washed, dried and observed under oil immersion objective lens after placing a drop of immersion oil. A green space with in the cells would indicate the presence of spores.

3.6.8.2 Biochemical characteristics

3.6.8.2.1 Catalase test

As stated in Cheesbrough (2006), the test identifies organisms that produce the enzyme catalase. A drop of 30 % freshly prepared hydrogen peroxide (3 ml H_2O_2 in 7 ml H_2O) was placed on a clean slide and loopful of isolate was transferred into it and emulsified. The appearance of gas bubbles indicates positive reaction. The reagent was shaken before the test to expel any dissolved oxygen and avoid a false positive result.

3.6.8.2.2 Indole test

As stated in Cheesbrough (2006), the tryptone-broth was prepared and 5 ml was dispensed into each test tubes and sterilized. The isolates were inoculated into the test tube and incubated at 28 $^{\circ}$C for 48 h. After incubation, 5 drops of Kovac's reagent (4 – p – dimethyl-amino benzaldehyde) were added to the tubes, shaken gently and allowed to settle. A red colouration in alcohol dye indicates a positive result for the reaction.

3.6.8.2.3 Motility test

A directional and purposeful movement of the organisms demonstrate motility. Nutrient broth was supplemented with 0.2 % agar, dispensed into test tubes and sterilized by autoclaving at 121 $^{\circ}$C and 15 psi for 15 minutes. The inoculated test tubes were incubated for 24 h. Diffused growth, which spreads throughout the medium, indicates motility. Non-motile organisms grew along the line of inoculation (Cheesbrough, 2006).

3.6.8.2.4 Methyl red-Voges Proskauer test

The separate test tubes containing MR-VP broth were inoculated with the isolates and incubated at 37 $^{\circ}$C for 24 - 48 h. After incubation, approximately 1/3rd of each cultures were transferred into empty glass test tubes and set aside for MR - VP test. Five drops of the methyl red indicator were added to the remaining broths in the original test tubes and colour change was observed. 12 drops of VP reagent 1 (5 % α - naphthol solution in absolute ethanol) were added to each of the broths that were set aside for the VP test. Each cultures were shaken to mix the reagent with the rest of the broths. Immediately, 2-3 drops of VP reagent 2 (40 % KOH in distilled water) were added and the cultures shaken again. The cultures were shaken again every 3-4 minutes until approximately 15 minutes had passed. Results were compared with the control (Cheesbrough, 2006).

3.6.8.2.5 Citrate test

As stated in Willey*et al*. (2008), the test was used to determine organisms that could utilize citrate as a sole-carbon source for metabolism. Slant of Simmon's citrate agar were prepared according to the manufacturer's instructions. The slants were inoculated by streaking over the surface with a loopful of an 18 h old culture and incubated at 37 °C for 48 h. Positive results were indicated by the growth on agar and a change in colour from green to blue and absence of colour change indicates a negative result.

3.6.8.2.6 Urease test

As stated in Cheesbrough (2006), the test determines the abilities of organisms to produce urease enzyme which breaks down urea (by hydrolysis) to give ammonia and CO_2. Test organisms were inoculated in test tubes containing 3 ml sterile Christensen's modified urea broth and incubated at 35 - 37 ºC for 24 h. Pink colour in the medium indicates positive result.

3.6.8.2.7 Starch hydrolysis test

To determine the abilities of the isolates to hydrolyze starch, 50 µl of liquid cultures of each isolates were dropped on starch-based solid medium containing per litre, 3 g meat extract, 10 g starch and 15 g agar (Cheesbrough, 2006).

3.6.8.2.8 Gelatin liquefaction test

As stated by Willey*et al*. (2008), gelatin agar medium was composed of 40 g/l of gelatin, 30 g/l of tryptic soy broth and 100 ml of distilled water. A small inocula of the isolates was stabbed to about three - quarter of the way to the bottom of a tube of deep agar with the

inoculating needle. The separate stab tubes for each of isolates were incubated at 37 $^{\circ}$C for 24 - 48 h. The incubated stab and the un-inoculated control tubes were placed into the refrigerator for approximately 30 minutes. The inoculated stab tubes were compared with the control by tapping the tubes gently.

3.6.8.2.9 Nitrate reduction test

As stated in Cheesbrough (2006), the test determines the ability of microorganisms to reduce nitrate to nitrite using a reductase enzyme. Nitrate broth was prepared by dissolving 30 g of sodium chloride (NaCl), 3.0 g meat extract, 5.0 g peptone and 1.0 g potassium nitrate (KNO_3) in 1000 ml of water. The broth was inoculated with the isolates in sterile test tubes and incubated for 48 h. Two nitrate reagents I and II were added to the test samples after incubation. A deep red colouration within 5 minutes is nitrate reductase positive. If no colour change is observed, the result is not conclusive. Then small amount of zinc is added to the broth. If the solution remains colourless, then both nitrate reductase and nitrite reductase is present. If the solution turns red, nitrate reductase is not present.

3.6.8.2.10 Coagulase test

As stated in Cheesbrough (2006), the test determines the abilities of organisms to produce the enzyme coagulase which causes plasma to clot by converting fibrinogen to fibrin. A loopful of each isolates were transferred into a drop of distilled water placed on slides and emulsified, followed by transfer of loopfuls of plasma cells to each of the suspensions and mixed gently. Clumping of the organisms within 10 seconds indicates a positive result.

3.6.8.2.11 Hydrogen sulphide production test

As stated in Willey*et al*. (2008), the test determines the abilities of the organisms to reduce sulfur compounds. Triple sugar iron agar slants were prepared and each isolates were inoculated into test tubes by streaking the inocula across the top of the slants and stabbing the slant tubes to the bottom. Tubes were incubated at 28 $^{\circ}$C for 24 h. Positive result is indicated by the formation of black colour coupled with displacement of the agar slant and red to yellow colour observation.

3.6.8.2.12 Sugar fermentation test

As stated in Willey*et al*. (2008), the test determines the abilities of the isolates to ferment glucose, sucrose, lactose, mannitol, maltose, xylose, arabinose and saccharose and also ability to produce gas. The fermentation medium contained 1 % peptone water and 5 drops of 0.2 % bromothymol blue indicator solution. Then, 9 ml of medium was dispensed into clean dry test tubes in which Durham tubes had been dropped (inverted and without air space) and sterilized by autoclaving at 121 $^{\circ}$C and 15 psi for 15 minutes. 1 ml of the sterile 5 % test sugar solution was added to medium and inoculated with a loopful of the test organisms and incubated at 30 $^{\circ}$C for 24 h. A change in colour of the medium (from blue to yellow) was recorded as positive reaction, while presence of gas in Durham tubes indicates gas production.

3.6.8.2.13 Oxidase test

As stated in Willey*et al*. (2008), the test identifies any organism that produces the enzyme oxidase. A loopful of isolates was transferred into pieces of Whatman No. 1 filter paper, impregnated with a solution of freshly prepared oxidase test reagent (N, N, N`, N` tetra-methyl-phenylene diamine) and smeared. Oxidation of the phenylene diamine in the reagent to dark purple or blue colour within 10 seconds indicates a positive result.

3.6.8.2.14 Casein hydrolysis test

The casein hydrolysis was observed by observing zones of clearing after 24 h of incubation. For this purpose, 50 µl liquid cultures of each isolates were dropped on casein-based solid medium containing (per litre) 10 g casein and 15 g agar. After 24 h of incubation, the inhibition zones were determined (Cheesbrough, 2006).

3.6.8.3 Molecular characteristics

3.6.8.3.1 Identification of bacteria

The Gramreaction test was first used to verify the morphological characteristics and purity of the nine marine aromatic – degrading bacterial strainsprior to molecular identification (Ubani *et al*., 2016).

3.6.8.3.2 Genomic DNA extraction of the bacterial isolates

DNA extraction was conducted using conventional method of Cetyltrimethyl Ammonium Bromide (CTAB) protocol in sterile Eppendorf tubes. About 10 ml of the pure cultures from nutrient broth was vortexed and 1.5 ml of it was transferred into 2 ml Eppendorf tubes and centrifuged with a microcentrifuge (Eppendorf Minispin plus, 12 x 1.5/2.0 ml) at 14,000 rpm for 5 minutes The supernatant was discarded to recover the pellets, which was then resuspended in a solution containing 567 µl of tris ethylene diamine tetraacetic acid buffer (tris EDTA or TE buffer), 30 µl of 10 % sodium dodecyl sulphate (SDS) and 3 µl of proteinase K (20 mg/ml) and was incubated in Accu block digital dry bath incubator (Labnet International, USA) at 65°C for 1 h. Then 180 µl of 5 M NaCl and 80 µl of 10 % CTAB solutions were added to the mixture and incubated for 10 minutes at 65°C. After which equal

volumes (400 ml) of phenol and chloroform was added to each tube and centrifuged at 14, 000 rpm for 15 minutes and then 300 µl of the supernatant was transferred into new sterile Eppendorf tubes and the DNA was precipitated by adding 0.6 ml cold isopropanol to each tube. The precipitate was collected by spinning the tube in a centrifuge at 14, 000 rpm for 15 minutes and the supernatant was discarded. Then 200 µl of freshly prepared 70 % ethanol was added to the tube to wash DNA pellets by spinning at 14, 000 rpm for 10 minutes. The supernatant was carefully removed to air - dry the DNA pellets and 100 µl of TE buffer was added to the dried DNA pellets and incubated at 37°C for 60 minutes to dissolve the DNA pellets. Then 1 µl of RNAase was added to the tube and incubated at 37°C for 60 minutes. The DNA was separated electrophoretically with 1 % agarose gel stained with 0.1 µg/ml ethidium bromide stain. The 20 cm long gels were running at 80 V/cm for 60 minutes in 100 ml of 1x tris acetate EDTA (TAE) electrophoretic buffer. The DNA was visualised by UV fluorescence to determine the success of the extraction process (Ubani *et al.*, 2016).

3.6.8.3.3 Polymerase chain reaction (PCR) and sequencing of the extracted pure DNA

The master mix aliquot for the PCR was dispensed into individual PCR tubes and the different DNA samples were added to each tubes. The negative control was used to check for contamination in the master mix. The PCR reagents in each tube amounted to 50 µl containing: buffer (5µl), $MgCl_2$ (1.5 µl), primer 1 (forward 16S - P1 PCR 5′AGAGTTTGATCCTGGCTCAG3′) (2 µl), primer 2 (reverse 16S-P2 PCR 5′AAGGAGGTGATCCAGCCGCA3′) (2 µl), dNTP mix (1µl), Dream Taq (0.25 µl), sterile sabax water (35.25 µl) and DNA samples (3 µl). The PCR reactions was performed using MJ Mini thermal cycler (Bio-Rad, Hercules, CA, USA). The cycling conditions was set at (a) initial denaturation 10 minutes at 95°C for 1 cycle. (b) Denaturation at 95 °C for 30 seconds, (c) Annealing cycling at 94°C for 30 seconds, (d) Elongation at 54 °C for 2 minutes. All steps

in denaturation, annealing and elongation was for 35 cycles and (e) final elongation 10 minutes at 72 °C for 1 cycle. The reaction was held at 4 °C for 1 h in the thermal cycler. The PCR products (genes) were separated at 1 kbp size and visualized as stated above. Then the PCR products (20 µl each) were cleaned up later using 160 µl of 13 % polyethylene glycol (PEG) 8000, 20 µl of 5 M NaCl solution and 200 µl of 70 % ethanol. The cleaned PCR products were sent for sequencing and was conducted using the automated DNA sequencer (Perkin-Elmer) in line with the sequencing kit protocol at the Forestry and Agricultural Biotechnology Institute (FABI) Sequencing Facility, University of Pretoria, South Africa (Ubani *et al.*,2016).

3.6.8.3.4 Blasting and phylogenetic correctional analysis

The blasting (BLAST) of DNA sequences was performed by revising the sequences of the 16S rDNA region obtained using BioEdit software, corrected sequences duplicated in a FASTA format form and completed in the National Centre for Biotechnology Information (NCBI) website. Homologies of the genes sequences were checked and compared with the sequences of those on the NCBI database and finally aligned using MAFFT software. The taxonomic correctional studies were done using Mega 7 software and evolutionary distance of the isolates were calculated using neighbour- joining (NJ) method. NCBI accession numbers were also assigned to the selected bacterial strains and the nucleotide sequence details of 16S rRNA genes of the isolates were already submitted in the GenBanknucleotide sequence databases. (Hesham *et al.*, 2014; Swaathy *et al.*, 2014a; Isiodu *et al.*, 2016; Ubani *et al.*, 2016).

3.6.9 Determination of concentration effect of aromatics on the growth of the isolates

In order to determine the concentration effects of the aromatic hydrocarbon compounds on the growth of the isolates, precisely 100 ml of modified mineral basal medium (MBM) was

dispensed into forty-five (45) 250 ml flasks and sterilized by autoclaving. The flasks were then divided into nine sets of seven flasks. Thereafter, 50, 100, 200 and 300 ppm levels of xylene, anthracene and pyrene which were seperately dissolved in acetone (as before) were exposed to each isolates. The fifth, sixth and seventh flasks served as the controls for each hydrocarbons and contained no xylene, anthracene and pyrene. Inoculated and control flasks were then incubated as previously described at 28 °C for 5 days. After incubation, five millilitre (5 ml) each sample was aseptically collected from each flask and assayed for the level of bacterial growth which was indicated by increase in turbidity of the medium measured at 600 nm using a UV-VISspectrophotometer (Astell UV- Vis Grating, 752 W) (John and Okpokwasili, 2012).

3.6.10 Substrate specificity test

In addition to growth on xylene, anthracene and pyrene, the purified strains were also tested for growth on other petroleum products and heavy metals. The petroleum products include: crude oil, diesel oil, kerosene, engine oil, benzene, toluene, hexane, paraben, ethyl benzene, and phenol while the heavy metals include include copper (II) chloride ($CuCl_2$), arsenic trioxide (AsO_3), lead (II) sulphate ($PbSO_4$), mercury (II) chloride $HgCl_2$, potassium dichromate ($K_2Cr_2O_7$), potassium iron cyanide (KFeCN), cadmium oxide (CdO), manganese (II) chloride ($MnCl_2$) and zinc sulphate ($ZnSO_4$). Modified mineral basal medium (MMBM) containing 1 % v/v of the above petroleum products were inoculated and incubated for 7 days at 28 °C while Seawater Nutrient medium (SWNM) containing 300 mg/l of the metals were inoculated and incubated for 5 days at 28 °C. After incubation, five millilitre samples were aseptically collected from each flask and assayed for the level of microbial growth which was indicated by increase in turbidity (OD density OD) of the medium measured in triplicate determination at 600 nm using a UV-VISspectrophotometer (Astell UV- Vis Grating, 752 W). Cultures without increase in turbidity over initial optical density and non-inoculated

control were scored as no growth (-) while cultures with increased turbidity significantly greater than the control (i.e. growth attenuation, optical density OD reading above 0.2) were scored as growth (+) (Jaysanker *et al.*, 2007; John and Okpokwasili, 2012).

3.6.11 Biodegradation study and analytical technique

Following the methods of Bennet *et al.* (2012) and John and Okpokwasili (2012) as modified in this study, the degradation rates of bacterial isolates were determined using hydrocarbon supplemented MMBM as previously stated.Precisely, 1 ml of 48 h old cultures of each bacteria was introduced into 28 sterile 200 ml capacity conical flasks (4 sets of 7 flasks) in triplicates containing 100 ml of sterile modified mineral basal medium supplemented with 100 mg/l of xylene, anthracene and pyrene hydrocarbons, respectively as source of carbon at 24 $^{\circ}$C for 24 days. During incubation, 5 ml representative samples from the different sets of flasks were pipetted at intervals of 0, 4, 8, 12, 16, 20 and 24 days and the residual hydrocarbons were determined spectrophotometrically using ethyl acetate as the extraction solvent. For each sample, 5 ml ethyl acetate was added and vigorously shaken manually. The organic and water layers from the media were separated by centrifugation at 5000 rpm for 20 minutes. The water layers were discarded while the organic layers were analyzed with UV-VIS spectrophotometer at 240 nm wavelength (Astell UV- Vis Grating, 752 W). The percentages of biodegradation of the hydrocarbons were determined as follows:

$$\% \text{ degradation } = \frac{a - b}{a} \times \frac{100}{1}$$

Where a = the absorbance of the medium before incubation; b is the maximum absorbance of the medium after each 4th day of the incubation period.

3.6.12 Identificationof metabolites formed/degraded products using instrumental analysis

3.6.12.1 Thin layer chromatographic (TLC) analysis for the degraded aromatic hydrocarbons

This was carried out using the method described by Bennet *et al*. (2012) as modified in this study to check whether the 100 mg/l of the aromatic hydrocarbons from the MBM as stated above were degraded and reduced into lesser and minor froms and parts by the existence of either small spots or by reduction in sizes in comparison to the controls. On a clean glass chamber, mixture of hexane/benzene: methanol at 25 ml and 5 ml proportion was prepared as solvent system for the chromatogram. One microliter (1 µl) of the organic phase was spotted and marked at the lower portion of the TLC plate. Similarly, control spots of xylene, anthracene and pyrene solutions with medium and standard hydrocarbons were placed. Plates were placed inside the glass chamber and were again covered. Once the solvent front reached the top layer of the plate, the developed chromatogram was removed and dried naturally on ambient temperature. It was then visualized first with UV light (235 nm) and lastly with iodine crystals. Test and control samples were compared from one another following the criteria of light intensity and spot sizes as a clear proof of biodegradation loss of some hydrocarbon components.

3.6.12.2 Gas chromatography – mass spectrometry (GC – MS) analysis

The polycyclic aromatic hydrocarbons present in the extracts were quantified using GC/MS as described by Bobak (2010). The stock standard was restek cat. No 8270-1 which contains semivolatile mix. It was purchased from Sigma Aldrich, South Africa. The concentration of the stock standard was 1000 ppm and it was used to prepare the calibration standards of 10 ppm, 30 ppm and 50 ppm. Working standard solution was prepared from the surrogate standard using dichloromethane. Calculation of the required concentrations was based on the chemical formula: $C_1V_1 = C_2V_2$; Where C_1 = Concentration of stock solution, C_2 =

Concentration to be made, V_1 = Volume to be determined, V_2 = Volume required. These standards were first analysed using the GC/MS to register a known retention time to match with each compound. The qualitative and quantitative analyses of the semivolatile compounds present in the sample extracts were carried out with the GC/MS Agilent 7860 GC system and 5975C MSD, equipped with a 7683B autosampler (Agilent Technologies, USA). The sampler syringe was 5.0 µl and splitless injection was 1.0 µl. The carrier gas used was helium 30 cm/second and at a constant flow rate of 1 ml/minute. The inlet, splitless, 260 ^{o}C, purge flow was 50 ml/minute at 0.5 minute and gas saver was at 80 ml/minute at 3 minutes. Inlet liner was the deactivated dual taper direct connect. The column was Agilent HP-5ms ultra inert 30 m x 0.25 mm x 0.25 µm film thickness. The oven program was started at 40 ^{o}C for 1 minute to 100 ^{o}C (15 ^{o}C/min), 10 ^{o}C/minute to 210 ^{o}C (1 minute), 5 ^{o}C/minute to 310 ^{o}C and it was held for 8 minutes. The detection was MSD source at 300 ^{o}C, quadrupole at 180 ^{o}C, transfer line at 290 ^{o}C, scan range 45 to 450 amu. The vials were amber screwed top glass vials and the vial cap was blue screwed cap. The vial inserts were 100 µl glass/polymer feet. The septum was advanced green. The ferrules were 0.4 mm id short; 85/15 vespel/graphite. The magnifier was 20X magnifier loupe. This instrument works on principle that a small amount of liquid extract injected into the instrument id volatilized at the hot injection chamber. The volatilized molecules are swept by a stream of inert carrier gas through a heated column that holds a high boiling liquid as the stationary phase. As the mixture flows along the column, the components bombard each other at different rates between the gaseous phase, dissolved in the high boiling liquid and it is then separated into pure components. The compounds are passed through a detector which sends an electronic signal to the recorder which responds by peak formations. The peaks formed are quantified by mass selective detector using the retention time of the relative compounds registered from a known standard. PAHs are identified by retention times matching to standards concentration. The value of the

chromatogram was quantified using peak area integration. The extraction procedure and extract analysis on all samples as well as the quality control and assurance of gas chromatographic – mass spectroscopic analyses were described by Bobak(2010) and the mass spectra obtained after the analysis were compared to the mass spectra in the the PubChem and NIST library/database (Kim *et al.*, 2016).

3.6.13 Detection of catabolic and surfactant genes by PCR analysis

3.6.13.1 PCR primer design

Following the method of Swaathy *et al.* (2014a) as modified in this study, a portion of catechol gene 881 bp (*C23O*) was pulled out from the genomic DNA using F: 5′- ATG AGC AAC AAA TAC GAA TT- 3′ and R: 5′- TCA AAC GGT CAA TCT GAT AT- 3′ primers. Likewise, according to the method of Qazi *et al*. (2013) and Swaathy *et al*. (2014b) as modified in this study, the primer pair of microsurf gene *srfA3/licA3F*: CAAAAKCGCAKCATATGAG and *srfA3/licA3R*: AGCGGCAYATATTGATGCGGYTC was designed to amplify a 881 bp portion of the *srfA3* or the homologous *licA3* gene present in surfactin/lichenysinand gene-specific primers of rhamnolipid gene (*rhlB*) using kpd-F 5'-GCCCACGACCAGTTCGAC-3' and kpd-R 5' CATCCCCCTCCCTATGAC-3'. They were subjected to the same PCR and gel electrophoretic conditions and protocols stated above (Ubani *et al.*,2016).

3.6.14 Investigation of the presence of the degradative bacterial genes on plasmid or chromosome

3.6.14.1 Plasmid curing experiment

Plasmid analysis was performed to show whether the hydrocarbon degrading and surfactant genes are plasmid - encoded or chromosomal - encoded by adopting the method of John and

Okpokwasili (2012) and Kumar *et al.* (2010). Zero point one millilitre of the culture of the MMBM supplemented with 100 mg/l of xylene, anthracene and pyrene hydrocarbons, respectively were added to the nine flasks containing 100 ml of nutrient broth (NB) containing ethidium bromide (450 µg/ml) for curing experiment. The set ups were incubated at 30 °C for 24 h. Thereafter, the broth was agitated to homogenize the content and loopful of the broth medium was subcultured on Nutrient agar (NA) plate and also on MBM agar containing the respective hydrocarbons. The plates were incubated at 37 °C for 24 h and the colonies counted. The colonies that are able to grow on NA but failed to grow on MBM agar plates were considered cured.

3.6.14.2 Screening for the presence of degradative bacterial genes on plasmid or chromosome using antimicrobial susceptibility testing

Antimicrobial susceptibility of bacterial isolates was performed on modified mineral basal agar (MBA) plates using a disc diffusion method in order to screen for the presence of degradative or surfactant bacterial genes on plasmid or chromosome. In this case, 0.1 ml of the 24 - 48 h cultures of the test isolates were pippeted, inoculated and spreaded in nine Petri dishes (90 x 15 mm) containing MBA agar and allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. Thereafter, commercial discs containing 15 µg each of levofloxacin, ampicillin, amoxicillin and cefadroxil; 25 µg each of tetracycline; 30 µg each of chloramphenicol, erythromycin and azithromycin (Anatech, South Africa) were aseptically placed on the surfaces of the sensitivity agar plates using sterile forceps and incubated at 37 °C for 24 h. After incubation, zones of inhibitions were observed against the entire antibiotics and recorded. The presence of zone of inhibition (sensitive colony) is indicative of chromosome-mediated resistance (plasmid not cured) while absence of zone of inhibition

(resistant colony) was indicative of plasmid-mediated resistance (plasmid cured). The cured strains were sub – cultured in Nutrient broth medium with the original hydrocarbons to confirm loss of degradation ability (John and Okpokwasili, 2012; Kumar *et al.*,2010).

3.6.14.3 Plasmid isolation and agarose gel electrophoresis

Plasmid DNAs were extracted by hot alkaline method for all plasmid sizes and bacteria with slight modifications both on the cured and uncured isolates.Two to three millilitres (2 - 3 ml) of the cultures was centrifuged, pellet resuspended in 1 ml of solution containing 0.04 M Tris-acetate, pH 8.0 (adjust pH with glacial acetic acid) and 2 mM EDTA. Two millilitres (2 ml) of lysis buffer (0.05 M Tris, 3 % SDS, pH 12.50, adjusted with 2 N NaOH) were added and mixed. The mixtures were incubated at 60 °C for 45 minutes. Four hundred microliters (400 µl) hot samples of phenol/chloroform (1:1) were added and mixed gently to complete emulsification. Phases were separated by centrifugation at 13, 400 x g for 15 minutes at room temperature and the upper aqueous phases were transfered carefully (avoid interphase which contains debris) to new tube containing 600 µl of chloroform. The supernatants were mixed and centrifuged again for separation of phases. The aqueous phases were recovered and used directly for agarose gel in order to check for the purity of the extracted plasmids as well as determine the number and size of the plasmids harbouring the catabolic genes involved in aromatic hydrocarbon degradation. The extracted plasmid (5 µl) and 250 ng of 1 kilo base pairs (1kbp) template supercoiled bacterial plasmid DNA ladder (Inqaba Biotech, SA) were each mixed with 5 µl of loading buffer and electrophoresed on a 0.7 % horizontal agarose gel at 50 V for 3 h. The gels were stained with ethidium bromide and bands were visualized with a UV transmilluminator(Kumar *et al.*, 2010; Mirdamadian *et al.*, 2010, Rhode and Henze, 2011; John and Okpokwasili, 2012). The 1 kbp supercoiled bacterial plasmid DNA ladder which serves as standard contains DNA fragments ranging from 100 bp to 10,000 bp. The

distance migrated by each of the plasmid DNA framents on the gel was measured. A calibration was constructed of the log size (bp) versus the respective distance migrated by the the DNA fragment. The sizes of the unknown plasmid DNA fragments of the experimental samples were determined using the method of Dohan (2005).

3.7 Analysis of Data

The data were analyzedusing Graph-Pad Prism statistical software version 7.00 (GraphPad software Inc. San Diego, California). All values were expressed as mean ± standard deviation (SD). Three types of analyses were performed:

1. Non- Linear regressional (curve fit) and linear probit model regressional analyses were used to determine the median effective concentrations (ErC_{50}, EC_{50}) and median lethal concentration (LC_{50}) of the toxicant samples relative to the control;
2. Ordinary one-way and two-way analyses of variance (ANOVA) for comparisons of differences among means of groups of the aromatic hydrocarbons, heavy metals, general parameters and the degradation efficacy of the aromatic hydrocarbons by each bacterial species followed bypost Tukey's, Dunnett's and Bonferroni's multiple comparison test with that of control;
3. Pearson correlation coefficient analysis to findthe effect relationships between the variables of toxicity and degradation assays.
4. The results were considered statistically significant and valid if the $p < 0.05$ and $CV \leq 25$ % (Nwinyi *et al*., 2013; Adenike, 2014; EBPI, 2016; Ichor *et al*., 2014; Meliani and Bensoltane, 2014; Morcos *et al*., 2015; Wanjohi *et al.*, 2015; Khoshnood et al. 2017).

CHAPTER FOUR

4.0 RESULTS

4.1 Physico-chemical Characteristics

4.1.1 Total aromatic hydrocarbon (TAH) contents

The result of the total aromatic hydrocarbon fractions (ppb) of sediment and water samples of the three sampling locations is presented in Table 4.1. From the result, there are more hydrocarbon fractions in sediment than water samples with anthracene (116.40 ± 0.12 ppb) and pyrene (5.90 ± 0.30 ppb) hydrocarbons having the highest fractions in Nembe sediment and Onne water samples, respectively while 1, 2-benzoanthracene as well as benzo (b) fluoranthene and acenaphthylene were not detected in sediment and water samples. There were no statistically significant differences detected using ordinary one-way ANOVA and Tukey's multiple comparison test among the treatment group of aromatic hydrocarbons and the sampled locations ($P > 0.05$) (Appendix VIa).

Table 4.1: Total aromatic hydrocarbon fractions (ppb) of sediment and water samples of the three sampled locations

Hydrocarbon	ABSE	NESE	ONSE	ABW	NEW	ONW
Acenaphthene	11.10 ±0.20	6.90 ± 0.20	9.70 ± 04.97	NA	5.10 ± 0.25	NA
Acenaphthylene	0.60 ± 0.20	0.40 ± 0.20	1.20 ± 0.25	NA	NA	NA
Phenanthrene	8.70 ± 0.12	11.60 ± 0.20	2.10 ± 0.25	02.20 ± 0.31	3.10 ± 0.25	2.00 ± 0.10
Anthracene	1.10 ± 0.27	116.40 ± 0.12	1.30 ± 04.40	NA	3.80 ± 0.25	NA
Flouranthene	1.10 ± 0.12	4.90 ± 0.25	1.30 ± 4.40	01.10 ± 0.15	3.10 ± 1.50	1.10 ± 0.20
Benzo(k)pyrene	3.10 ± 0.25	NA	3.10 ± 0.30	01.50 ± 0.20	NA	1.20 ± 0.20
Benzo(a)pyrene	0.60 ± 0.12	0.20 ± 0.31	0.40 ± 2.00	0.10 ± 0.02	0.30 ± 0.02	0.20 ± 0.10
Xylene	58.90 ± 0.21	103.60 ± 0.20	6.20 ± 2.00	1.70 ± 0.20	NA	1.80 ± 0.02
Benzo(b) flouranthene	5.00 ± 0.03	2.60 ± 0.20	3.60 ± 0.20	NA	NA	NA
Pyrene	3.50 ± 0.24	40.50 ± 0.20	3.70 ± 0.20	03.90 ± 0.20	NA	5.90 ± 0.30
Benzo (g,h,i) perylene	8.60 ± 0.20	12.00 ± 0.20	8.40 ± 0.25	03.00 ± 0.20	1.10 ± 0.20	3.00 ± 0.25
Dibenzyl (a,h) anthracene	3.00 ± 0.20	4.80 ± 0.25	4.30 ± 0.25	Nd	4.00 ± 0.21	NA

1,2-benzoanthracene	NA	7.70 ± 0.25	NA	NA	NA	NA
Flourene	NA	11.20 ± 0.20	NA	01.10 ± 0.02	0.80 ± 0.02	1.10 ± 0.25

KEY: ONSE= Onne sediment; ABSE = Abonema sediment; NESE = Nembe sediment; ONW = Onne water, ABW = Abonema water, NEW = Nembe water; NA = Not available (below detectable limit); ppb = part per billion; values are mean ± standard deviation of triplicate determination; Detection limit: PAH = 0.0001 µg/g; xylene, anthracene and naphthalene = 0.002 µg/ml.

4.1.2 Total heavy metal concentrations

The result of the total heavy metal contents (ppm) of sediment and water samples of the three sampled locations is presented in Table 4.2. From the result, there are more heavy metal contents in sediment than water samples with iron metal having the highest fractions in both Abonema sediment (110.24 ± 0.20 ppm) and water (3.27 ± 0.25 ppm) samples, respectively. The order of concentration abundance of these metals in sediments from the three locations is in the sequence: Fe > Zn > Cu > Ni > Cr > Hg > Cd > Co > Pb > As while the order of concentration abundance of these metals in water from the three locations is in the sequence: Fe > Zn > Hg > Ni > Cd > Co > Cr > Cu > Pb > As. There were no statistically significant differences detected using ordinary one-way ANOVA and Tukey's multiple comparison test among the treatment group of heavy metals and the sampled locations (P > 0.05) (Appendix VIb).

Table 4.2: Total heavy metal contents (ppm) of sediment and water samples of the three sampled locations

Metals	ABSE	NESE	ONSE	ABW	NEW	ONW
Iron (Fe)	110.24 ± 0.20	72.02 ± 0.12	104.44 ± 0.23	03.27 ± 0.25	01.11 ± 0.105	02.20 ± 0.20
Cobalt (Co)	0.13 ± 0.03	ND	0.05 ± 0.02	0.05 ± 0.02	ND	ND
Copper (Cu)	0.79 ± 0.122	0.20 ± 0.02	01.31 ± 0.12	ND	ND	ND
Lead (Pb)	0.48 ± 0.24	ND	ND	ND	ND	ND
Cadmium (Cd)	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	ND
Chromium (Cr)	0.58 ± 0.02	0.16 ± 0.02	0.31 ± 0.02	0.02 ± 0.02	ND	ND
Zinc (Zn)	11.72 ± 0.20	2.64 ± 0.02	4.14 ± 0.02	0.06 ± 0.02	0.13 ± 0.00	0.10 ± 0.01
Nickel (Ni)	0.58 ± 0.02	0.32 ± 0.02	0.3 ± 0.00	ND	0.11 ± 0.02	0.05 ± 0.02

Mercury (Hg)	0.14 ± 0.02	0.34 ± 0.02	0.10 ± 0.01	0.13 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
Arsenic (As)	ND	ND	ND	ND	ND	ND

KEY: ONSE = Onne sediment; ABSE = Abonema sediment; NESE = Nembe sediment; ONW = Onne water, ABW = Abonema water, NEW = Nembe water; ppm = part per million; values are mean ± standard deviation of triplicate determination; ND = Not detected; Detection limit: Fe, Co, Pb, Cd, Cr, Ni and As = 0.0002 ppm; Cu and Zn = 0.0001 ppm; Hg = 0.0005 ppm.

4.1.3 General parameter profile

The result of the general parameter profiles of the sediment and water samples of the three sampled locations are presented in Tables 4.3 and 4.4. From the tables, conductivity at 25 °C (EC25) for Onne, Abonema and Nembe sediments are 2.41 ± 0.25 dS/cm, 2.06 ± 0.31 dS/cm and 1.99 ± 0.02 dS/cm while Onne, Abonema and Nembe waters are 6.99 ± 0.27 dS/cm, 1.97 ± 0.03 dS/cm and 1.32 ± 0.13 dS/cm, respectvely. Onne sediment and water samples exhibited lower pH values (6.72 ± 0.02 and 6.41 ± 0.02) followed by Abonema (7.47 ± 0.02 and 6.55 ± 0.02) and then Nembe (7.16 ± 0.02 and 7.31 ± 0.16), respectively. All the sediment samples had greater percentage of sand followed by clay and then silt with Onne sediment having the highest sand percentage of 59.72 ± 0.02 %. Grain size measurements of superficial sediments revealed that Abonema and Nembe contained clayey loam whereas Onne contained sandy loam. Nembe had the highest moisture content, porosity, bulk density and total organic carbon of 85.94 ± 33.11 %, 0.04 ± 0.00, 1.72 ± 0.00 g/ml and 17.82 ± 0.03 % and lowest soil saturation time of 18.00 ± 0.03 minutes which contained clayey loam sediment type. Nembe had highest total nitrogen (TN) of 3.81 ± 0.01 % and 0.97 ± 0.01 % whereas Onne and Abonema had highest total phosphorus (TP), potassium (K) and calcium (Ca) content of 5.50 ± 0.00 mg/l and 3.72 ± 0.02 mg/l; 11.45 ± 0.00

ppp and 4.60 ± 0.01 ppm; and 12.78 ± 0.02 ppm and 6.30 ± 0.01 ppm for both sediment and water samples, respectively. Furthermore, Abonema water having the highest COD (106.60 ± 65.56 mg/l), DO (25.30 ± 0.08 mg/l), BOD (210.00 ± 2.52 mg/ml), TDS (7.48 ± 0.01 mg/l), TSS (0.15 ± 0.00 mg/l) and TS (7.63 ± 0.01 mg) followed by Nembe and Onne water samples. The COD and BOD values were found to be higher than WHO standards except TDS, TSS and TS that were found below the maximum recommended limits. There were no statistically significant differences detected using ordinary one-way ANOVA and Tukey's multiple comparison test among the treatment group of the general parameters of the sediment and water samplesof the three sampled locations ($P > 0.05$) (Appendices VIc and VId).

Table 4.3: General parameterprofile of sediment samples of the three sampled locations

Parameters	Sediment sampling locations		
	Abonema	Nembe	Onne
Conductivity at 25 % (dS/cm)	2.060 ± 0.31	1.99 ± 0.02	2.41 ± 0.25
pH	7.47 ± 0.02	7.16 ± 0.02	6.72 ± 0.02
Sand (%)	43.79 ± 0.25	47.63 ± 0.02	59.72 ± 0.02
Clay (%)	32.59 ± 0.05	26.51 ± 0.00	18.56 ± 0.05
Silt (%)	23.62 ± 0.23	25.94 ± 0.04	21.72 ± 0.01
Moisture (%)	49.75 ± 19.01	85.94 ± 33.11	21.80 ± 0.30
Porosity	0.04 ± 0.00	0.04 ± 0.00	0.01 ± 0.00
Bulk density (g/ml)	1.36 ± 0.00	1.72 ± 0.00	1.58 ± 0.00
TN (%)	3.30 ± 0.00	3.80 ± 0.01	2.69 ± 0.00
TP (mg/l)	5.33 ± 0.00	4.70 ± 0.10	5.50 ± 0.00
Potassium (ppm)	9.24 ± 0.02	8.45 ± 0.03	11.45 ± 0.00
Calcium (ppm)	10.68 ± 0.01	11.99 ± 0.01	12.78 ± 0.02
Soil saturation (min.)	22.48 ± 0.03	18.00 ± 0.31	28.32 ± 0.02

TOC (%)	12.65 ± 0.05	17.82 ± 0.03	14.56 ± 0.03
Sediment type	Clay loam	Clay loam	Sandy loam

KEY: TN = Total nitrogen; TP = Total phosphorus; TOC = Total organic carbon

Table 4.4: General parameterprofile of water samples of the three sampled locations

Parameters	Water sampling locations		
	Abonema	Nembe	Onne
Conductivity at 25 % (dS/cm)	1.97 ± 0.03	1.32 ± 0.13	6.99 ± 0.27
pH	6.55 ± 0.02	7.31 ± 0.16	6.41 ± 0.02
TDS (mg/l)	7.48 ± 0.01	6.56 ± 0.02	5.76 ± 0.62
TSS (mg/l)	0.15 ± 0.00	0.01 ± 0.00	0.07 ± 0.00
TS (mg)	7.63 ± 0.01	6.56 ± 0.00	5.83 ± 0.21
COD (mg/l)	106.60 ± 65.56	80.00 ± 46.84	66.67 ± 31.41
DO (mg/l)	22.50 ± 0.05	25.30 ± 0.08	19.30 ± 0.15
BOD (mg/l)	210.00 ± 2.52	146.00 ± 0.20	46.00 ± 0.20
TN (%)	0.28 ± 0.01	0.97 ± 0.01	0.55 ± 0.03
TP (mg/l)	3.72 ± 0.02	2.16 ± 0.01	2.07 ± 0.02
Potassium (ppm)	4.64 ± 0.01	3.88 ± 0.01	4.28 ± 0.00
Calcium (ppm)	6.35 ± 0.01	5.78 ± 0.00	4.10 ± 0.00

KEY: TDS = Total dissolved solid; TSS = Total suspended solids; TS = Total solids; COD = Chemical oxygen demand; DO = Dissolved oxygen; BOD = Biological oxygen demand; TN = Total nitrogen; TP = Total phosphorus

4.2 Acute Toxicity Profile

4.2.1 Marine algal toxicity profile

The results of the mean values of cell algal density measurements (cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of the aromatic hydrocarbon - contaminated distilled water and sediment samples during 0 -72 has well as features of the marine toxicity test with microalga are presented in Appendices IIa, IIb, IIc, IId and IIe. From the Appendices, there were exponential growth patterns in the control test cultures ($\mu = 0.083\ h^{-1}$) while continuous inhibitions were observed at the varying test concentrations of the test samples.

The result of the inhibition of growth rate of *Phaeodactylum tricornutum* by aromatic hydrocarbon - contaminated distilled water and sediment of the three sampled locations is shown in Figure 4.1. From the result, there were drastic reductions in their respective specific growth rates leading to remarkable increase in the percentage inhibition values of all the test concentrations of the test samples with pyrene Nembe sediment having the highest inhibition of 98.19 ± 0.07 % and $\mu = 0.200\ h^{-1}$ during 0 – 72 h.

Moreso, the result of the mean 72 h ErC_{50} (mg/l) toxic response of *Phaeodactylum tricornutum* to aromatic hydrocarbon - contaminated distilled water and sediment of the three sampled locationsis shown in Figure 4.2. From the figure, positive control ($K_2Cr_2O_7$) had the highest EC_{50} value of 8.07 ± 0.03 mg/l with CV and r^2 values of 68.61 % and 0.99 while pyrene Nembe sediment had the least EC_{50} value of 4.63 ± 0.01 mg/l with CV and r^2 values of 78.27 % and 0.98 with very strong significant positive linear relationship between algal number and sample concentrations ($P < 0.05$) (Appendix VIe). There were no statistically significant differences detected using ordinary one-way ANOVA and Dunnett's multiple comparison test among the treatment group of aromatic hydrocarbons, sediment samples and the positive control ($P > 0.05$) (Appendix VIf).

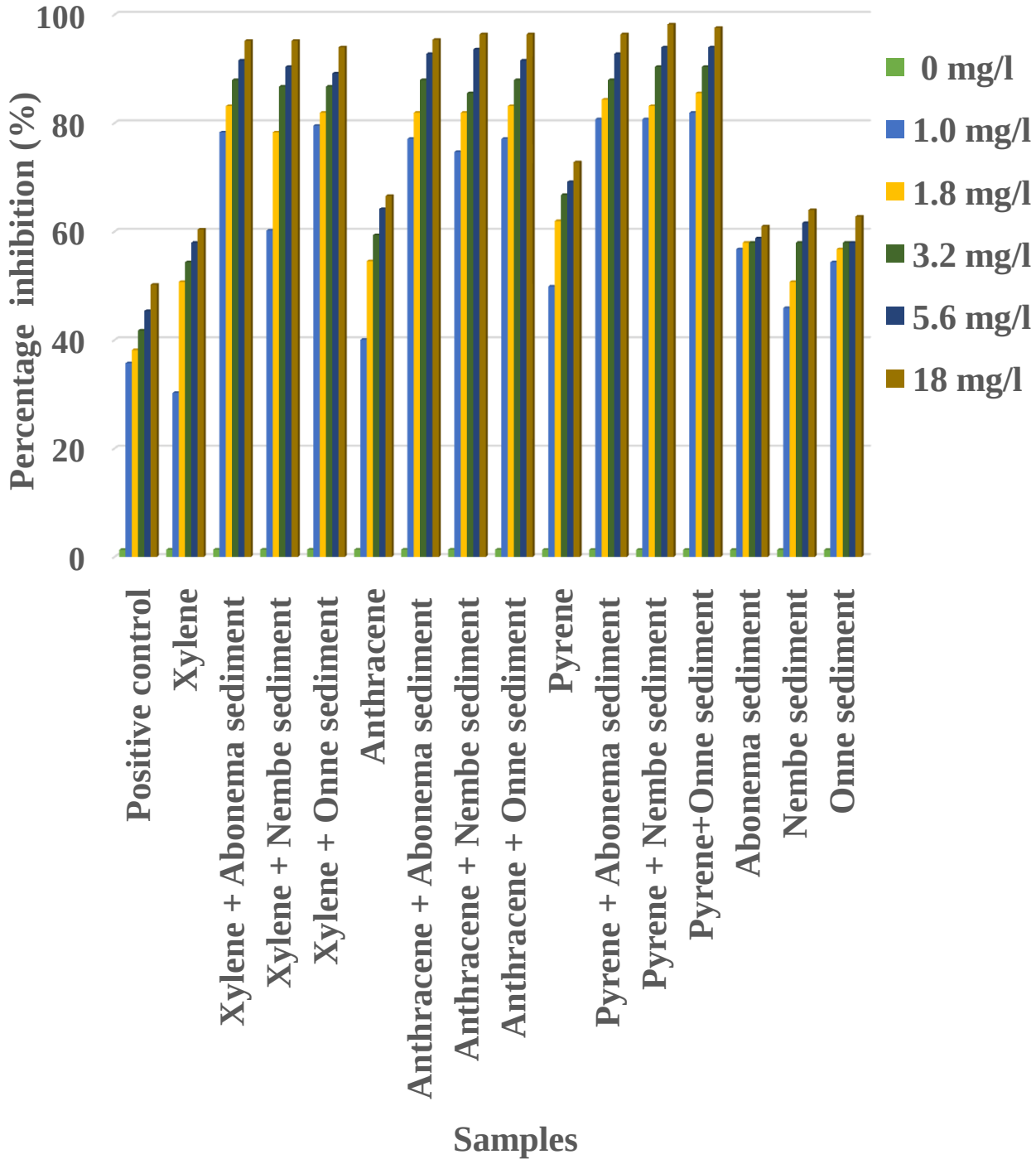


Figure 4.1: Inhibition of growth rate of *Phaeodactylum tricornutum* by aromatic hydrocarbon - contaminated distilled water and sediment of the three sampled locations

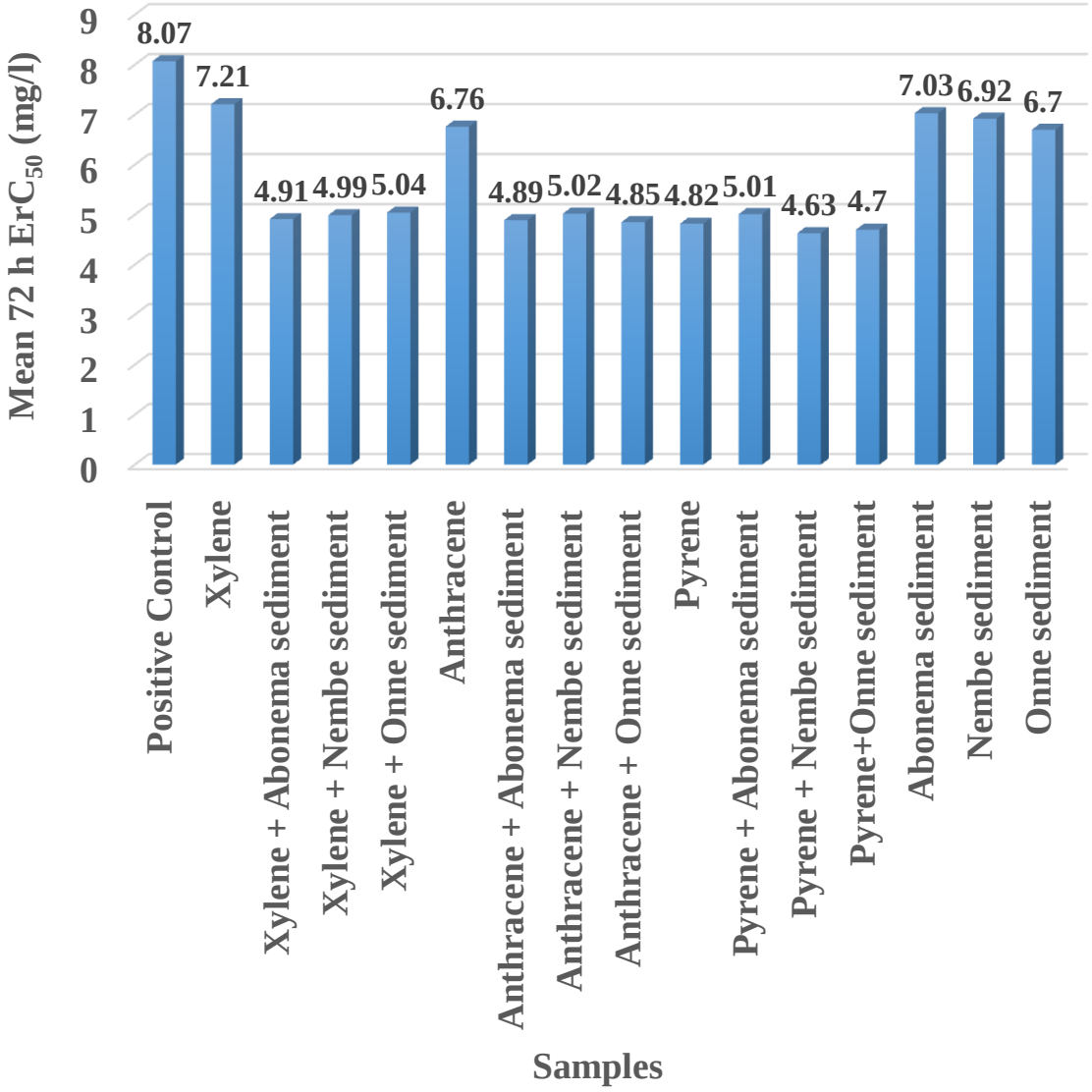


Figure 4.2: Mean 72 h ErC_{50} (mg/l) toxic response of *Phaeodactylum tricornutum* to aromatic hydrocarbon - contaminated distilled water and sediment of the three sampled locations

4.2.2 Phototoxicity profile

The result of the germination index (GI) of *Sinapsis alba*(mustard seeds) grown on the aromatic hydrocarbon - contaminated distilled water and sediment of the three sampled locations as well as the test concept of the phytotoxicity are presented on Appendices IIf, IIg and shown in Figure 4.3. From the results, the soils contaminated with aromatic hydrocarbons and sediments samples showed the lower GI values while individual test samples showed higher GI values. The most inhibitory effect was produced by pyrene + Onne sediment sample with GI and CV values of 7.14 ± 0.023 % and 0.37 % while the least inhibitory effect was produced by xylene + distilled water sample with GI and CV values of 28.57 ± 0.03 % and 0.18 %. The result also revealed that over 80.00 ± 0.00 % of seeds in the negative control samples had a mean germination success with more than 20 mm mean root length.There were statistically significant differences detected using ordinary one-way ANOVA and Dunnett's multiple comparison test among the treatment group of aromatic hydrocarbons, sediment samples and the positive control ($P < 0.05$) (Appendix VIg).

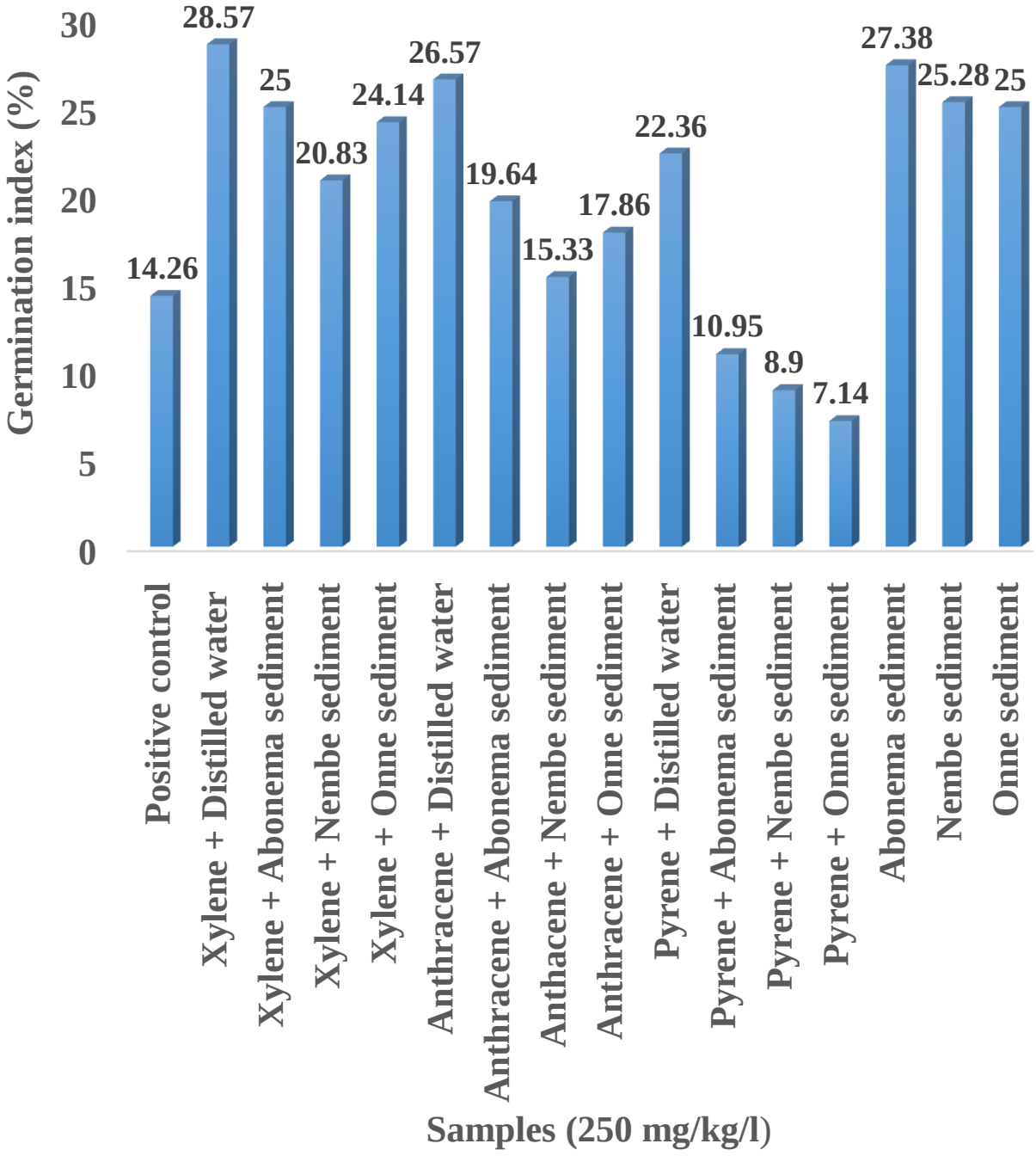


Figure 4.3: Germination index (GI) of *Sinapsis alba* (mustard seeds) grown on the aromatic hydrocarbon - contaminated distilled water and sediment of the three sampled locations

4.2.3 *Artemia* toxicity profile

The result of the percentage mortality scores of *Artemia franciscana* (brine shrimp)exposed to aromatic hydrocarbon - contaminated distilled water and waste water samples from the three sampled locations as well as the features of the *Artemia* toxicity screening test are presented and shown in Appendices IIh, IIiand Figure 4.4.

The result of the mean 24 h LC_{50} (mg/l) toxic response of *Artemia franciscana*(brine shrimp)to aromatic hydrocarbon - contaminated distilled water and wastewater of the three sampled locations is shown in Figure 4.5.

From the results, pyrene + Onne wastewater had the highest mortality and least LC_{50} scores of 93.33 ± 0.16 % and 30.34 ± 0.14 mg/l with CV and r^2 of 90.65 % and 0.96 while Abonema sediment had the least mortality and LC_{50} scores of 60.00 ± 0.14 % and 42.46 ± 0.19 mg/l with CV and r^2 of 102.84 % and 0.97 after 24 h of incubationwith very strong significant positive correlation between increasing test concentrations (0 – 100 mg/l) and *Artemia* percentage mortality scores (% dead) of the marine crustacean (Appendix VIh).The result also revealed that less than 06.67 ± 0.00 % of mortality in the negative control samples was observed which corresponds to EBPI (2016) guideline on biological validity criteria (< 10) of the assay. There were no significant differences detected using ordinary one-way ANOVA and Bonferroni's multiple comparisons test among the treatment group of aromatic hydrocarbon wastewaters and the positive control ($P > 0.05$) (Appendix VIi).

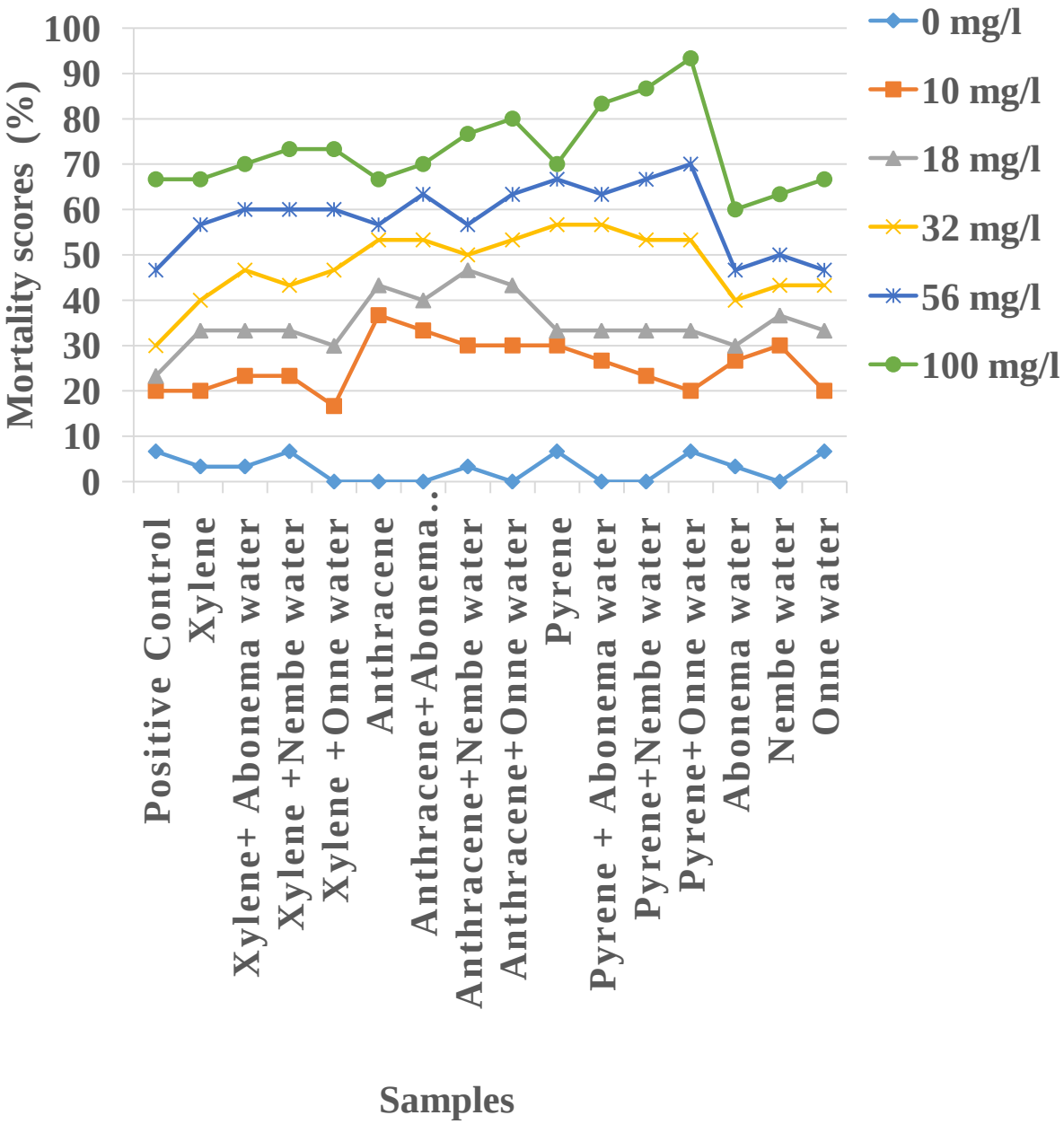


Figure 4.4: Percentage mortality scores of *Artemia franciscana* (brine shrimp)exposed to aromatic hydrocarbon - contaminated distilled water and wastewater from the three sampled locations

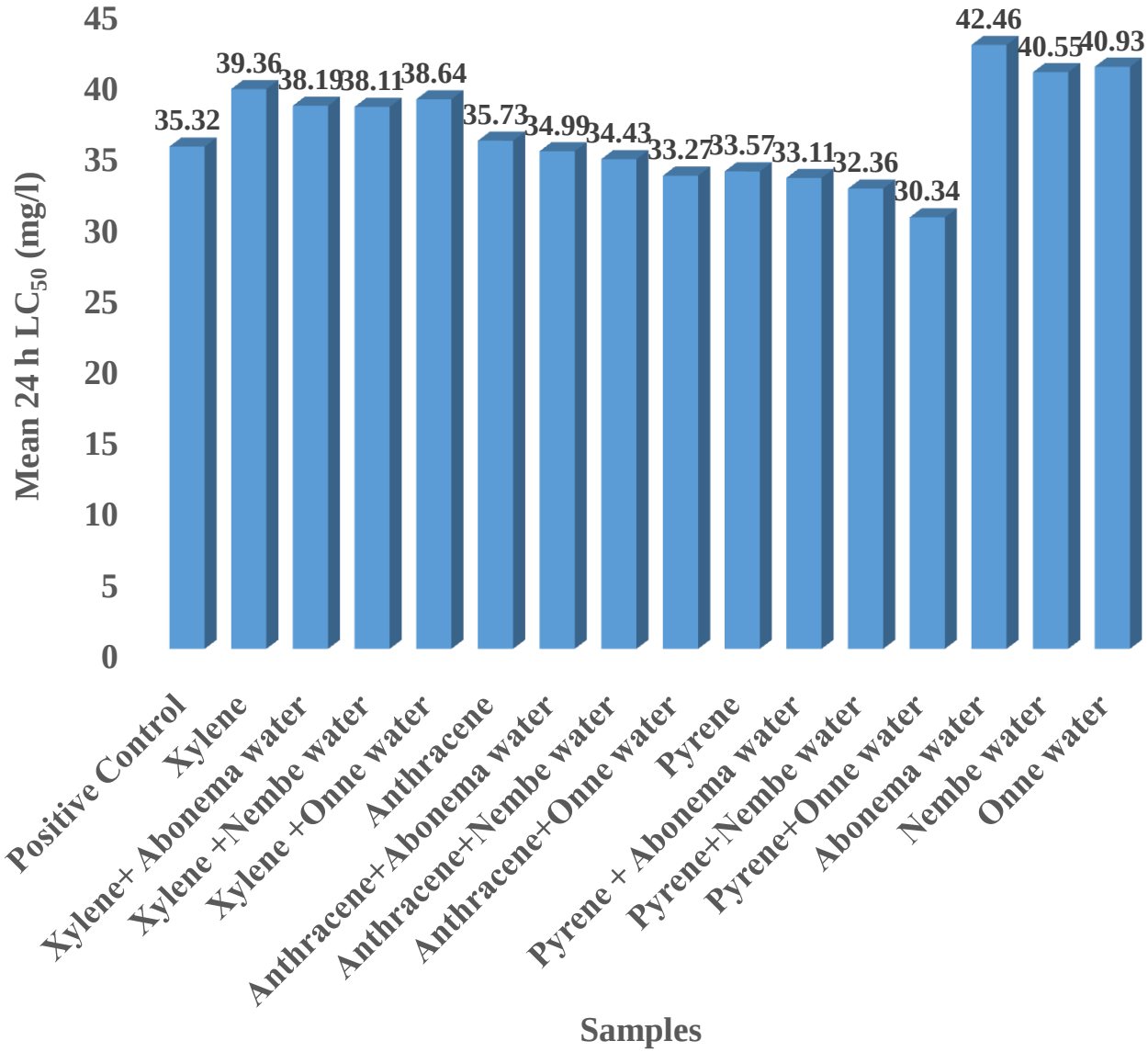


Figure 4.5: Mean 24 h LC_{50} (mg/l) toxic response of *Artemia franciscana* (brine shrimp) to aromatic hydrocarbon - contaminated distilled water and wastewater of the three sampled locations

4.2.4 Toxi – chromo profile

The result of the absorbance values of the toxicity of the aromatic hydrocarbons in distilled water and wastewater of the three sampled locations using Toxi-Chromotest at different dilutions and as well as microplate test result for Toxi - chromo testing of aromatic hydrocarbon compoundsare presented in Appendices IIj and IIk. From the result, pyrene and xylene hydrocabons in distilled water had the highest and lowest absorbance values of 0.398 ± 0.002 and 0.097 ± 0.002 as indicated in red figures respectively. The result of the toxicity factor (TF) of the aromatic hydrocarbons in distilled water and wastewater of the three sampled locations is shown in Figure 4.6. From the Figure, pyrene in Nembe water had the highest toxicity factorof 273.200 ± 0.163with CV and r^2 of 17.320 % and 0.097 and xylene in distilled waterhad the lowest toxicity factorof 07.300 ± 0.170with CV and r^2 of 7.980 % and 0.256showing no significant ($P > 0.05$) weak positive correlation respectively (Appendix VIj). The toxicity factor of aromatic hydrocarbon contaminated wastewaters were generally more than hydrocarbon - contaminated distilled water.

The result of the mean 1.5 h EC_{50} (mg/l) toxic response of mutant *E. coli* to the aromatic hydrocarbons in distilled and wastewater samples is shown in Figure 4.7. From the figure, xylene in distilled water had the highest EC_{50} of 3.417 ± 0.094 mg/l while pyrene Onne water had the least EC_{50} of 0.015 ± 0.002 mg/l. Generally, it could be deduced that pyrene and anthracene are significantly ($P < 0.05$) highly toxic aromatic hydrocarbons (EC_{50}< 1 mg/L) while xylene is a significantly ($P <0.05$) toxic aromatic hydrocarbon (1 mg /L<$EC_{50} \leq$ 10 mg /L) compared to the positive control ($HgCl_2$) (EC_{50}< 1 mg/L).There were statistically significant differences detected using ordinary one-way ANOVA and Dunnett's multiple comparison test among the means of the treatment group of aromatic hydrocarbons, wastewater and the control ($P <0.05$) (Appendix VIk).

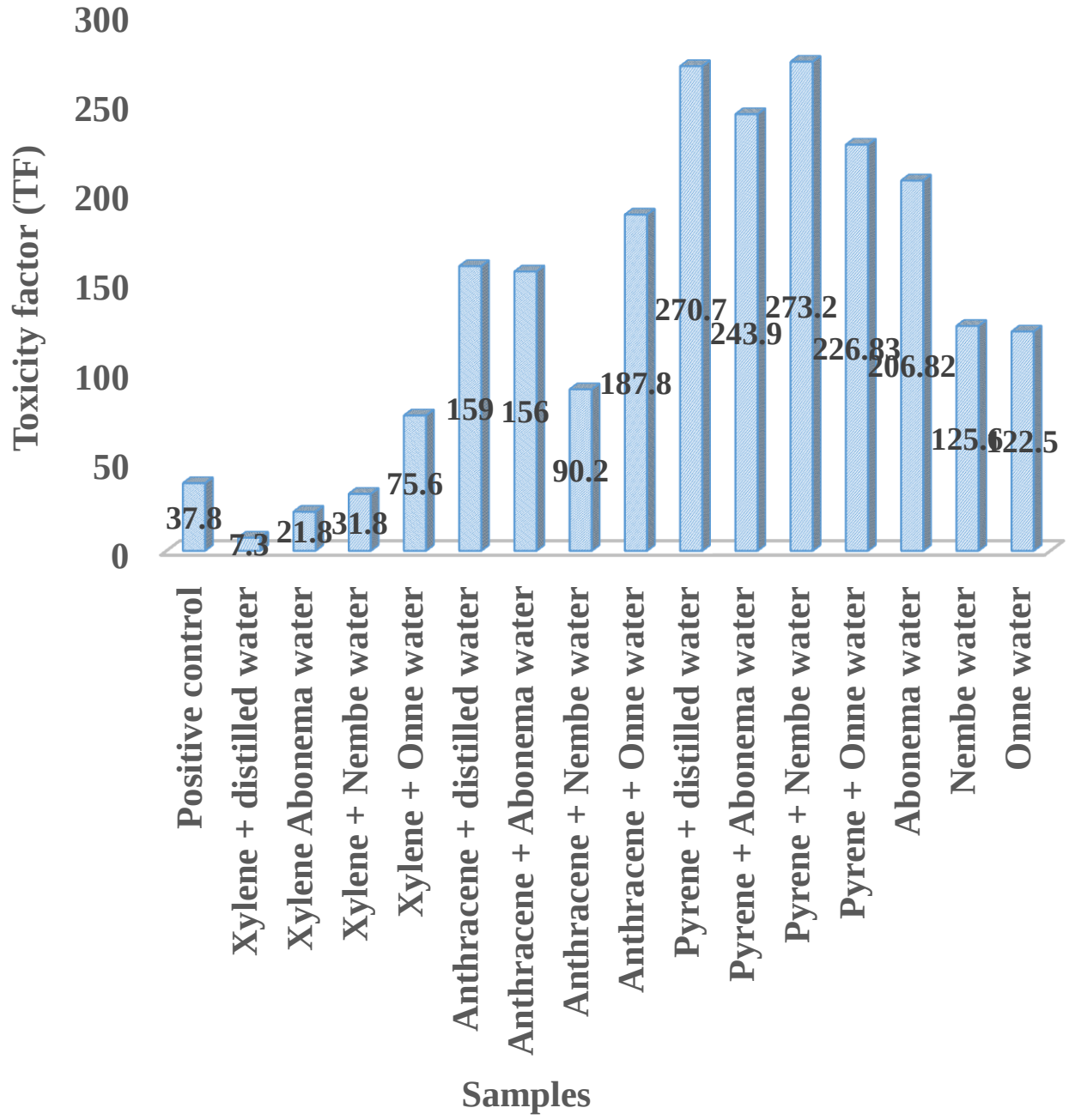


Figure 4.6: Toxicity factor (TF) of the aromatic hydrocarbons in distilled water and wastewater of the three sampled locations

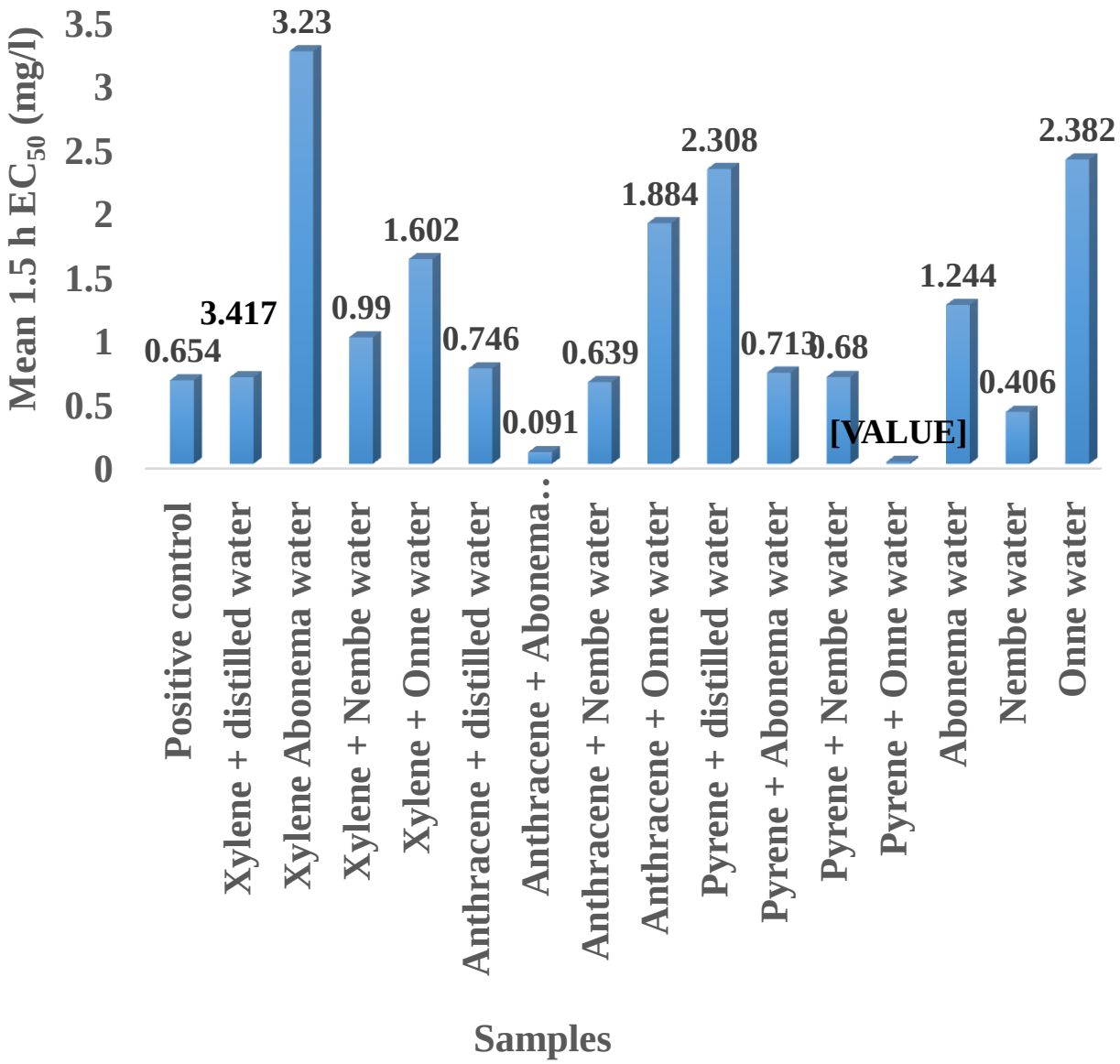


Figure 4.7: Mean 1.5 h EC_{50} (mg/l) toxic response of mutant *E. coli* to the aromatic hydrocarbons in distilled and wastewater samples

4.3 Sub - Chronic Health Profile

4.3.1 Body and organ weight outline

The result of the average body weight (g) of aromatic hydrocarbon - treated Wistar albino mice before and after 35 days of oral exposure is presented in Table 4.5. From the result, xylene 1000 mg/kg had the highest body weight of 27.87 ± 1.11 g before exposure and control without hydrocarbon had the highest body weight of 26.44 ± 0.91 g after exposure.

The results of the organ/tissue weight of xylene, anthracene, pyrene and benzo(a) pyrene - treated Wistar albino mice after 35 days' exposure are shown in Figures 4.8, 4.9, 4.10 and 4.11. From the results, increase in dose of xylene led to decrease in weight of stomach and liver from 0.93 ± 0.01 – 0.49 ± 0.03 g, while kidney and lung increase in weight from 0.17 ± 0.08 – 0.28 ± 0.01 g respectively. It was observed that increase in dose of anthracene led to decrease and increase in weight of stomach and lung from 0.68 ± 0.01 – 0.15 ± 0.01 g while liver and kidney increase and decrease in weight from 0.12 ± 0.01 – 0.31 ± 0.01 g respectively; increase in dose of pyrene led to decrease and increase in weight of stomach, liver and kidney from 1.49 ± 0.01 – 0.29 ± 0.01 g while lung increase and decrease in weight from 0.15 ± 0.01 – 0.20 ± 0.01 g respectively and finally increase in dose of benzo (a) pyrene led to increase and decrease in weight of stomach, liver and lung from 0.08 ± 0.01 – 2.74 ± 0.01 g while kidney increase in weight from 0.17 ± 0.01 – 0.24 ± 0.01 g, respectively. There were no statistically significant differences detected using two - tailed paired T- Test, repeated measures one-way ANOVA and Dunnett's multiple comparison test among the time of exposure and means of body/organ weight of the treatment group of aromatic hydrocarbons and their controls ($P > 0.05$) (Appendices VIl – VIm).

Table 4.5: Average body weight of aromatic hydrocarbon - treated Wistar albino mice before and after 35 days of oral exposure

	Average body weight	
Dose (mg/kg)	Before (g)	After (g)
	Control	
0	27.11 ± 0.28	26.44 ± 0.91
	Xylene	
400	24.13 ± 1.11	23.26 ± 0.51
1000	27.87 ± 1.11	21.81 ± 0.56
2000	24.62 ± 0.57	22.93 ± 0.89
	Anthracene	
100	23.21 ± 0.94	21.12 ± 0.34
500	25.10 ± 0.65	24.01 ± 0.39
1000	26.40 ± 1.34	26.37 ± 1.71
	Pyrene	
50	23.12 ± 0.40	20.88 ± 0.37
125	23.30 ± 0.35	19.43 ± 0.04
250	23.30 ± 0.35	17.88 ± 0.09
	Benzo (a) pyrene	
20	20.20 ± 0.32	20.19 ± 0.40
40	21.50 ± 0.13	20.77 ± 0.21
100	23.78±0.18	20.00 ± 0.33

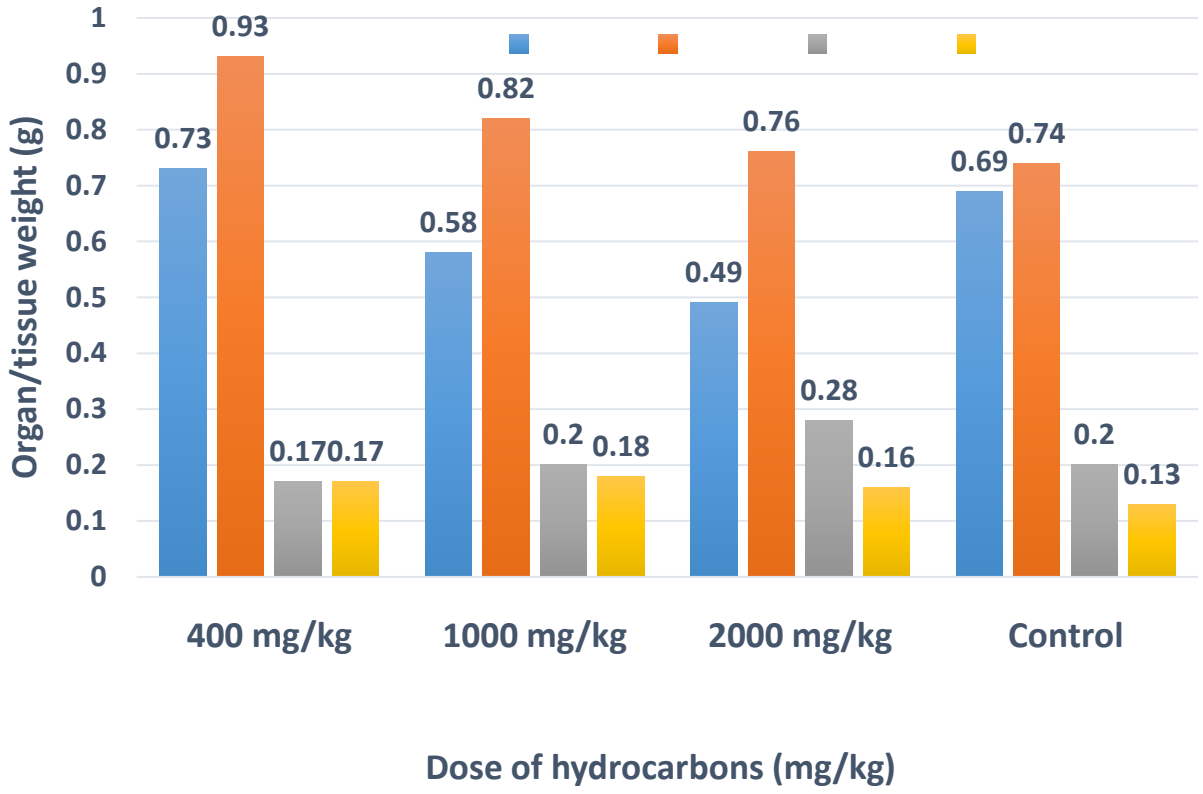


Figure 4.8: Organ/tissue weight of xylene - treated Wistar albino mice after 35 days' exposure

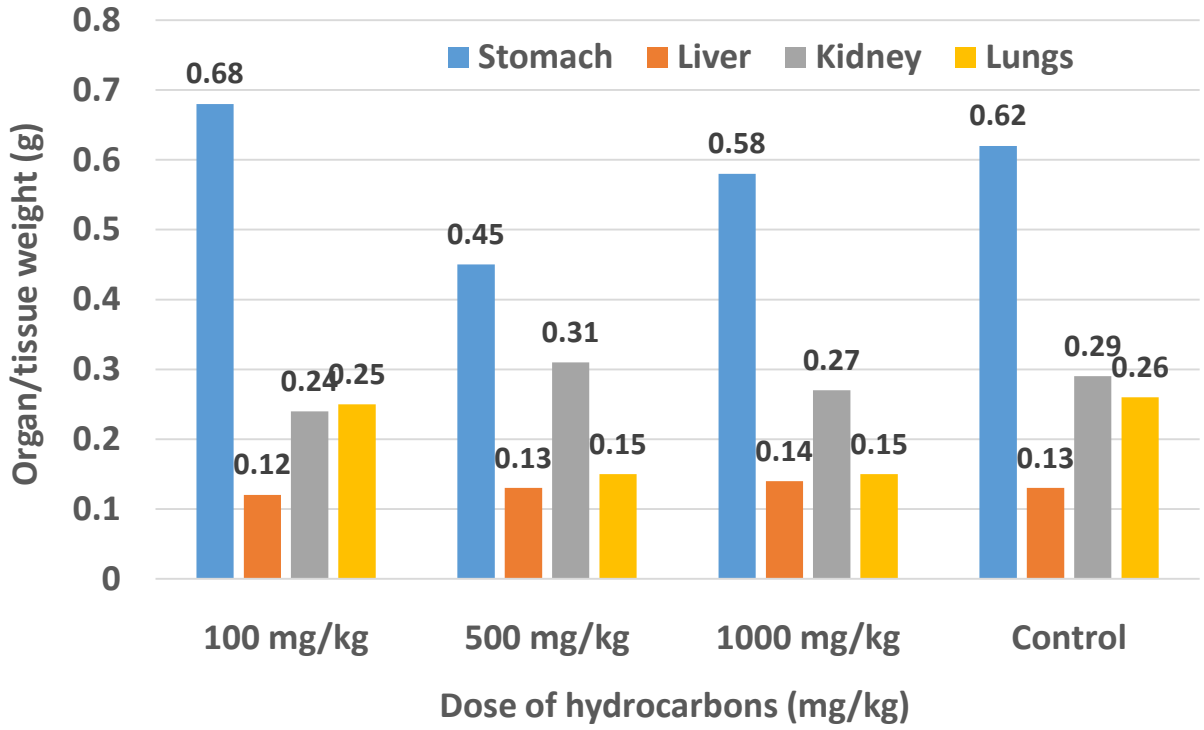


Figure 4.9: Organ/tissue weight of anthracene - treated Wistar albino mice after 35 days' exposure

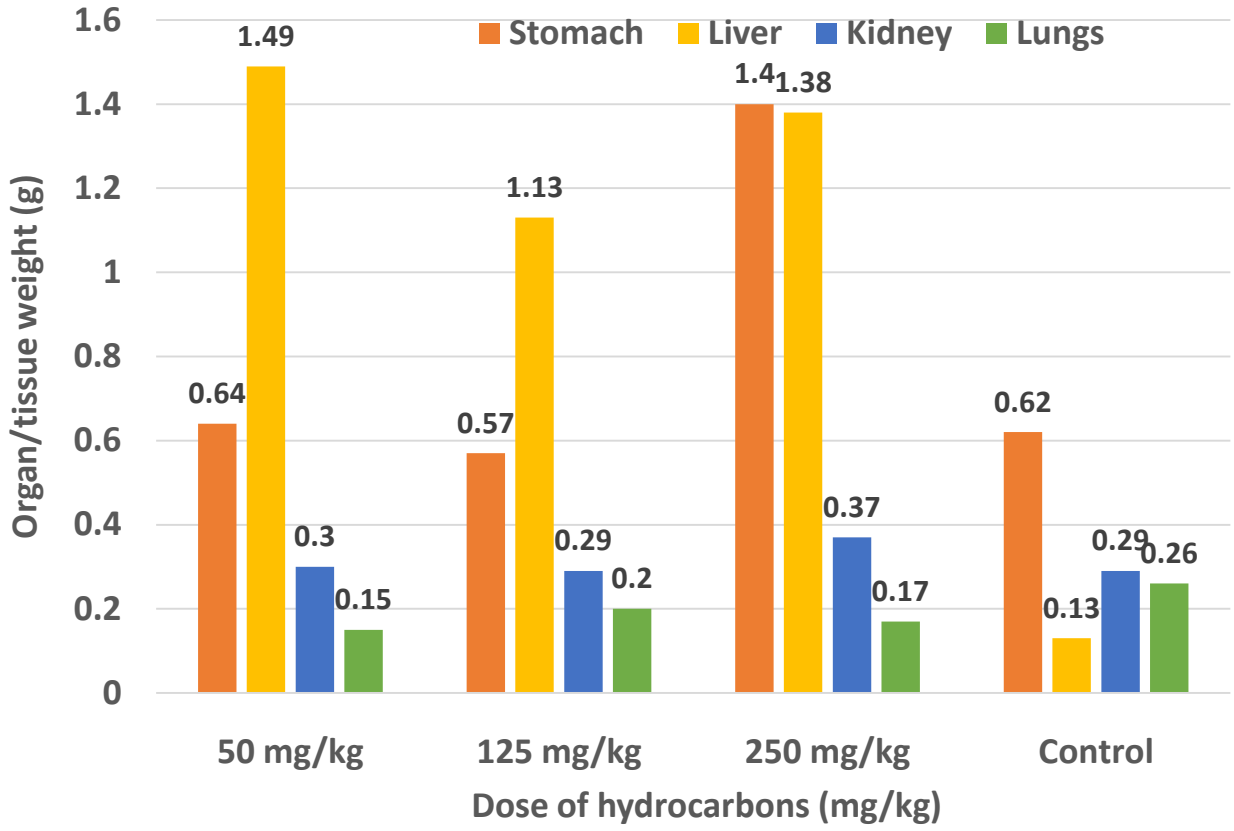


Figure 4.10: Organ/tissue weight of pyrene - treated Wistar albino mice after 35 days' exposure

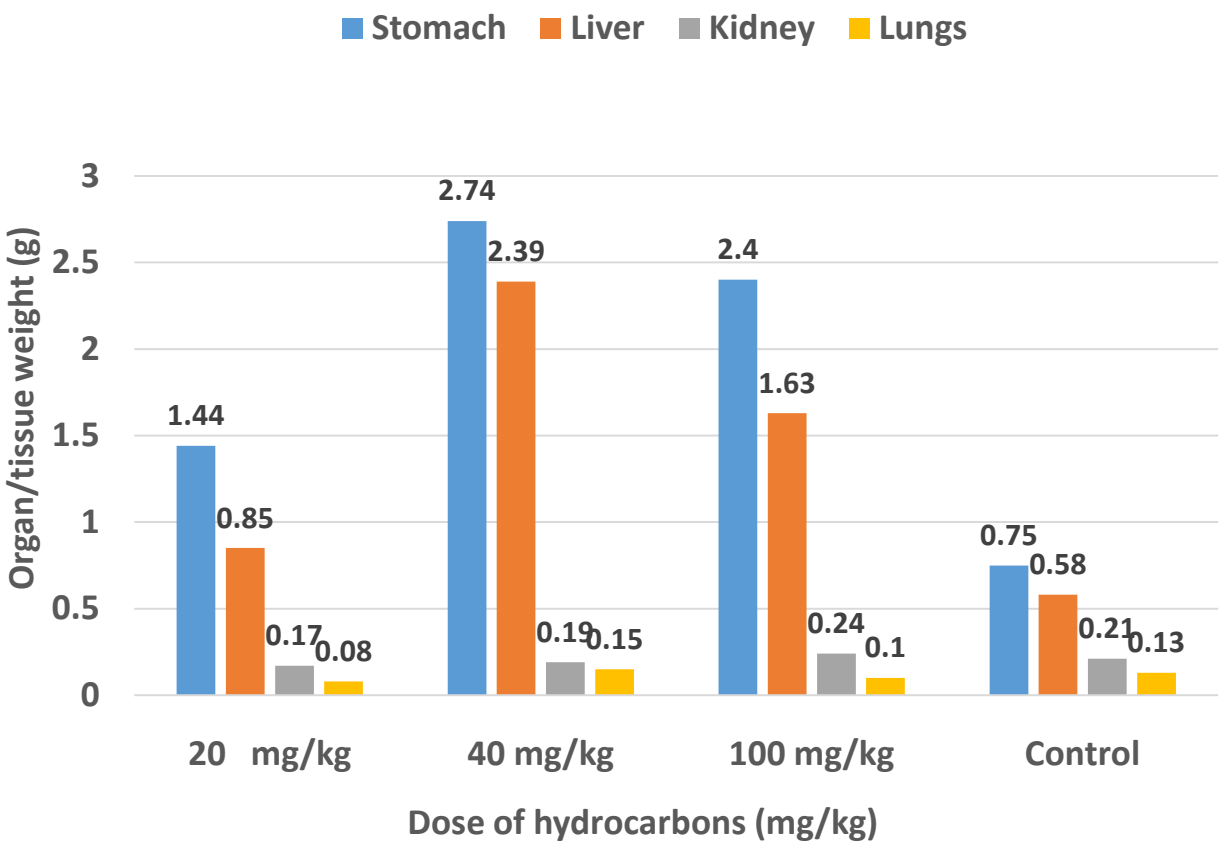


Figure 4.11: Organ/tissue weight of benzo (a) pyrene - treated Wistar albino mice after 35 days' exposure

4.3.2 Haematologicalprofile

The results of the effect of xylene, anthracene, pyrene and benzo (a) pyrene on haematological indices in Wistar albino mice at different doses after 35 days' exposure are presented on Tables 4.6, 4.7, 4.8 and 4.9. From the results, there were reductions in RBC (5.10 ± 0.13 - 4.00 ± 0.13 /l), Hb (14.40 ± 0.13 - 12.10 ± 0.16 g/dl), PCV (46.40 ± 0.16 - 36.00 ± 0.17 %), monocytes (10.40 ± 0.16 - 6.00 ± 0.16 %), basophils (2.0 ± 0.81 - 0.00 ± 0.00 %), decrease and increase in platelets and lymphocytes; increase and decrease in WBC, neutrophils and eosinophils as the dose/ concentration of xylene increased from 400 – 2000 mg/kg. There were reductions in RBC (7.80 ± 0.13 - 3.60 ± 0.13 /l) Hb (14.60 ± 0.13 - 11.00 ± 0.16 g/dl), PCV (46.90 ± 0.16 - 33.00 ± 0.17 %) and platelets (739.00 ± 0.16 - 185.00 ± 0.16 /l); increase in lymphocytes (32.00 ± 0.16 - 58.00 ± 0.13 %) and basophils (1.20 ± 0.81 - 1.00 ± 0.13 %), decrease and increase in WBC and neutrophils; increase and decrease in monocytes and eosinophils as the dose/ concentration of anthracene increase from 100 – 1000 mg/kg. There were reductions in RBC (5.50 ± 0.50 - 4.60 ± 0.50 /l), Hb (16.50 ± 0.50 - 13.80 ± 0.60 g/dl), PCV (51.00 ± 0.60 - 42.60 ± 0.70 %) and platelets (88.50 ± 0.50 - 13.50 ± 0.60 /l); increase in monoocytes (1.00 ± 0.40 - 5.80 ± 0.50 %) and basophils (0.00 ± 0.00 - 1.00 ± 0.40 %), decrease and increase in WBC, eosinophils and lymphocytes; increase and decrease in neutrophils and as the dose/ concentration of pyrene increased from 50 – 250 mg/kg. There were reductions in WBC (5.40 ± 0.17 - 4.70 ± 0.08 / l), RBC (4.70 ± 0.21 - 4.00 ± 0.35 /l), Hb (14.20 ± 0.13 - 12.10 ± 0.13 g/dl), platelets (526.00 ± 0.16 – 218.00 ± 0.13 /l), monocytes (8.00 ± 0.17 - 3.00 ± 0.21 %), eosinophils (3.00 ± 0.16 - 1.00 ± 0.13 %) and basophils (1.00 ± 0.22 - 0.00 ± 0.00 %); decrease and increase in neutrophils and PCV; increase and decrease in lymphocytes and as the dose/ concentration of benzo (a) pyrene increased from 20 – 100 mg/kg. The control values were within the reference range but higher or lower than the

treatment groups. There were extreme significant differences detected using two - way ANOVA and Dunnett's multiple comparison test among the dose effect of the treatment group of aromatic hydrocarbons and their controls ($P < 0.05$) but not significant among the haematological parameters ($P > 0.05$) (Appendix VIn).

Table 4.6: Effect of xylene on haematological indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	400 mg/kg	1000 mg/kg	2000 mg/kg
WBC (/L)	5.80 ± 0.50	4.60± 0.13	5.00 ± 0.13	2.30 ± 0.13
RBC (/L)	3.30 ± 0.60	5.10 ± 0.13	4.20 ± 0.13	4.00 ± 0.13
Hb (g/dL)	9.90 ± 0.70	14.40 ± 0.13	12.00 ± 0.16	12.10 ± 0.16
PCV (%)	30.00 ± 0.20	46.40 ± 0.16	37.80 ± 0.08	36.00 ± 0.17
Platelets (/L)	185.00 ± 0.30	421.00 ± 0.16	198.00 ± 0.13	401.00 ± 0.16
Neutrophils (%)	23.00 ± 0.70	19.90 ± 0.17	24.10 ± 0.14	17.20 ± 0.16
Lymphocytes (%)	60.00 ± 0.50	61.70 ± 0.16	51.30 ± 0.14	71.60 ± 0.13
Monocytes (%)	12.00 ± 0.40	10.40 ± 0.16	8.20 ± 0.16	6.00 ± 0.16
Eosinophil (%)	5.00 ± 0.70	6.00 ± 0.17	16.40 ± 0.16	5.20 ± 0.13
Basophils (%)	0.00 ± 0.00	2.00 ± 0.81	0.00 ± 0.00	0.00 ± 0.00

KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

Table 4.7: Effect of anthracene on haematological indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	100 mg/kg	500 mg/kg	1000 mg/kg
WBC (/L)	5.80 ± 0.50	9.50 ± 0.13	3.80 ± 0.13	5.60 ± 0.13
RBC (/L)	3.30 ± 0.60	7.80 ± 0.13	3.90 ± 0.13	3.60 ± 0.13
Hb (g/dL)	9.90 ± 0.70	14.60 ± 0.13	11.00 ± 0.16	11.00 ± 0.16
PCV (%)	30.00 ± 0.20	46.90 ± 0.16	35.20 ± 0.08	33.00 ± 0.17
Platelets (/L)	185.00 ± 0.30	739.00 ± 0.16	264.00 ± 0.13	185.00 ± 0.16
Neutrophils (%)	23.00 ± 0.70	58.00 ± 0.17	19.30 ± 0.14	24.00 ± 0.16
Lymphocytes (%)	60.00 ± 0.50	32.00 ± 0.16	56.30 ± 0.14	58.00 ± 0.13
Monocytes (%)	12.00 ± 0.40	7.00 ± 0.16	8.20 ± 0.16	6.00 ± 0.16
Eosinophil (%)	5.00 ± 0.70	3.00 ± 0.17	15.40 ± 0.16	11.00 ± 0.13
Basophils (%)	0.00 ± 0.00	1.00 ± 0.81	1.00 ± 0.81	1.00 ± 0.13

KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

Table 4.8: Effect of pyrene on haematological indices in Wistar albino miceat different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	50 mg/kg	120 mg/kg	250 mg/kg
WBC (/L)	5.80 ± 0.50	5.30 ± 0.40	2.30 ± 0.40	2.90 ± 0.50
RBC (/L)	3.30 ± 0.60	5.50 ± 0.50	4.60 ± 0.50	4.60 ± 0.50
Hb (g/dL)	9.90 ± 0.70	16.50 ± 0.50	13.90 ± 0.50	13.80 ± 0.60
PCV (%)	30.00 ± 0.20	51.00 ± 0.60	42.00 ± 0.40	42.60 ± 0.70
Platelets (%)	185.00± 0.30	88.50 ± 0.50	53.30 ± 0.50	13.50 ± 0.60
Neutrophils (%)	23.00 ± 0.70	30.00 ± 0.20	40.00 ± 0.50	30.00 ± 0.20
Lymphocytes (%)	60.00 ± 0.50	68.60 ± 0.50	54.20 ± 0.60	60.00 ± 0.50
Monocytes (%)	12.00 ± 0.40	1.00 ± 0.40	3.80 ± 0.80	5.80 ± 0.50
Eosinophils (%)	5.00 ± 0.70	0.40 ± 0.10	2.00 ± 0.60	3.00 ± 0.80
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.40

KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

Table 4.9: Effect of benzo (a) pyrene on haematological indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	20 mg/kg	40 mg/kg	100 mg/kg
WBC (/L)	5.80 ± 0.50	5.40 ± 0.17	4.70 ± 0.18	4.70 ± 0.08
RBC (/L)	3.30 ± 0.60	4.70 ± 0.21	4.00 ± 0.21	4.00 ± 0.35
Hb (g/dL)	9.90 ± 0.70	14.20 ± 0.13	12.10 ± 0.26	12.10 ± 0.13
PCV (%)	30.00 ± 0.20	42.00 ± 0.17	33.90 ± 0.14	36.40 ± 0.17
Platelets (/L)	185.00 ± 0.30	526.00 ± 0.16	416.00 ± 0.17	218.00 ± 0.13
Neutrophils (%)	23.00 ± 0.70	40.00 ± 0.13	25.00 ± 0.16	30.00 ± 0.17
Lymphocytes (%)	60.00 ± 0.50	48.00 ± 0.13	67.00 ± 0.17	65.10 ± 0.17
Monocytes (%)	12.00 ± 0.40	8.00 ± 0.17	5.00 ± 0.17	3.00 ± 0.21
Eosinophils (%)	5.00 ± 0.70	3.00 ± 0.16	3.00 ± 0.08	1.00 ± 0.13
Basophils (%)	0.00 ± 0.00	1.00 ± 0.22	0.00 ± 0.00	0.00 ± 0.00

KEY: WBC = White blood count; RBC = Red blood count; Hb = Haemoglobin; PCV = Packed cell volume

4.3.3 Biochemical characteristics

The results of the effect of xylene, anthracene, pyrene and benzo (a) pyrene on the biochemical indices in Wistar albino mice at different doses after 35 days'exposure are presented on Tables 4.10, 4.11,4.12 and 4.13. From the results, toxic effects of xylene were observed in all doses with maximum harmful effect at the highest concentration (2000 mg/kg) for ALT (26.20 ± 1.86 u/l), AST (28.70 ± 2.58 u/l), ALP (132.10 ± 0.26 u/l), total protein (15.50 ± 2.58 g/dl) and albumin (6.30 ± 2.58 g/dl) on the liver while maximum harmful effect at the lowest concentration (400 mg/kg) for the electrolytes and median concentration (1000 mg/kg) for creatinine (0.61 ± 1.29 mg/dl) and urea (19.30 ± 2.58 mg/dl) on the kidney. For anthracene, toxic effects were observed in all doses with maximum harmful effect at the highest concentration (1000 mg/kg) for total bilirubin (14.80 ± 0.90 mg/dl), direct bilirubin 97.20 ± 0.34 mg/dl), ALT (64.00 ± 2.45 u/l), AST (58.00 ± 2.45 u/l), total protein (6.60 ± 0.33 g/dl), albumin (37.90 ± 2.17 g/dl), creatinine (0.63 ± 0.32 mg/dl), urea (79.50 ± 2.13 mg/dl), potassium (4.90 ± 0.37 mmol/l), sodium (132 ± 2.94 mmol/l) and chloride (98.70 ± 0.29 mmol/l) on both the liver and kidney. For pyrene, toxic effects were observed in all doses with maximum harmful effect at the highest concentration (250 mg/kg) for total bilirubin (13.00 ± 0.10 mg/dl), direct bilirubin (4.90 ± 0.10 mg/dl), ALT (33.00 ± 0.34 u/l), total protein (9.70 ± 0.20 g/dl), sodium (138.20 ± 0.40 mmol/l); median concentration (125 mg/kg) for creatinine (0.40 ± 0.10 mg/dl), urea (65.00 ± 0.30 mg/l), potassium (6.20 ± 0.40 mmol/l) and lowest concentration (50 mg/kg) on AST (30.00 ± 0.30 u/l), ALP (312.00 ± 0.50 u/l), albumin (4.90 ± 0.60 g/dl), chloride (106.00 ± 0.30 mmol/l) on both the liver and kidney. For benzo (a) pyrene, toxic effects were observed in all doses with maximum harmful effect at the highest concentration (100 mg/kg) for total bilirubin (13.90 ±

0.13 mg/dl), ALT (56.00 ± 0.16 u/l), AST (60.80 ± 0.13 u/l), ALP (302.00 ± 0.17 ul), total protein (11.60 ± 0.13 g/dl), albumin (7.40 ± 0.16 g/dl), urea (72.60 ± 0.13 mg/dl), sodium (141.00 ± 0.16 mmol/l) and lowest concentration for direct bilirubin (6.40 ± 0.16 mg/dl) and potassium (4.10 ± 0.17 mmol/l) on both the liver and kidney. There was extreme significant dose effect by two - way ANOVA and Dunnett's multiple comparison test among the dose effect of the treatment group of aromatic hydrocarbons and their controls ($P<0.05$) but not significant among the haematological parameters ($P > 0.05$) (Appendix VIo).

Table 4.10: Effect of xylene on biochemical indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	400 mg/kg	1000 mg/kg	2000 mg/kg
Total bilirubin (total bili) (mg/dL)	10.90 ± 0.40	18.20 ± 0.04	11.92 ± 2.08	14.40 ± 0.13
Direct bilirubin (direct bili) (mg/dL)	4.80 ± 0.20	7.00 ± 0.13	6.30 ± 0.13	6.92 ± 0.22
Alanine transaminase (ALT) (U/L)	10.20 ± 0.20	8.10 ± 2.58	13.10 ± 0.13	26.20 ± 1.86
Aspartate transaminase (AST) (U/L)	9.00 ± 0.20	10.00 ± 2.58	11.60 ± 2.58	28.70 ± 2.58
Alkaline phosphatase (ALP) (U/L)	111.90 ± 0.20	88.70 ± 0.13	103.90 ± 2.58	132.10 ± 0.26
Total protein (TP) (g/dL)	9.30 ± 0.20	13.20 ± 0.16	11.92 ± 2.08	15.50 ± 2.58
Albumin (ALB) (g/dL)	4.30 ± 0.80	5.50 ± 0.13	6.30 ± 0.13	6.30 ± 2.58
Creatinine (CRE) (mg/dL)	0.20 ± 0.10	0.31 ± 2.58	0.61 ± 1.29	0.49 ± 1.29
Urea (UR) (mg/dL)	12.90 ± 0.60	16.60 ± 0.13	19.30 ± 2.58	15.00 ± 2.58
Potassium (K) (mmol/L)	3.30 ± 0.30	2.60 ± 0.13	2.80 ± 2.58	4.00 ± 0.13
Sodium (Na) (mmol/L)	130.20 ± 0.40	137.00 ± 0.13	136.10 ± 2.58	128.00 ± 2.58
Chloride (Cl) (mmol/L)	97.00 ± 0.20	116.00 ± 0.13	93.70 ± 2.58	100.60 ± 2.58
Bicarbonate (HCO_3) (mmol/L)	18.10 ± 0.30	19.40 ± 0.13	17.30 ± 0.13	19.40 ± 0.13

Table 4.11: Effect of anthracene on biochemical indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	100 mg/kg	500 mg/kg	1000 mg/kg
Total bilirubin (total bili) (mg/dL)	10.90 ± 0.40	8.80 ± 0.34	8.80 ± 0.37	14.80 ± 0.90
Direct bilirubin (direct bili) (mg/dL)	4.80 ± 0.20	5.20 ± 0.25	5.20 ± 0.29	7.20 ± 0.34
Alanine transaminase (ALT) (U/L)	10.20 ± 0.20	15.20 ± 0.25	14.10 ± 0.24	64.00 ± 2.45
Aspartate transaminase (AST) (U/L)	9.00 ± 0.20	10.10 ± 0.21	16.60 ± 0.57	58.00 ± 2.45
Alkaline phosphatase (ALP) (U/L)	111.90 ± 0.20	103.90 ± 1.00	122.00 ± 2.10	121.00 ± 0.24
Total protein (TP) (g/dL)	9.30 ± 0.20	4.80 ± 0.25	4.60 ± 0.33	6.60 ± 0.33
Albumin (ALB) (g/dL)	4.30 ± 0.80	11.00 ± 0.25	12.80 ± 0.37	37.90 ± 2.17
Creatinine (CRE) (mg/dL)	0.20 ± 0.10	0.28 ± 0.03	0.32 ± 0.05	0.63 ± 0.32
Urea (UR) (mg/dL)	12.90 ± 0.60	13.30 ± 0.33	14.40 ± 0.25	79.50 ± 2.13
Potassium (K) (mmol/L)	3.30 ± 0.30	4.00 ± 0.25	2.91 ± 0.37	4.90 ± 0.37
Sodium (Na) (mmol/L)	130.20 ± 0.40	131.10 ± 1.90	132.00 ± 1.18	132.00 ± 2.94
Chloride (Cl) (mmol/L)	97.00 ± 0.20	97.00 ± 0.37	89.00 ± 0.29	98.70 ± 0.29
Bicarbonate (HCO_3) (mmol/L)	18.10 ± 0.30	20.10 ± 0.25	18.80 ± 0.37	16.50 ± 0.37

Table 4.12: Effect of pyrene on biochemical indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	50 mg/kg	125 mg/kg	250 mg/kg
Total bilirubin (total bili) (mg/dL)	10.90 ± 0.40	8.50 ± 0.50	10.60 ± 0.30	13.00 ± 0.10
Direct bilirubin (direct bili) (mg/dL)	4.80 ± 0.20	3.70 ± 0.20	4.80 ± 0.20	4.90 ± 0.10
Alanine transaminase (ALT) (U/L)	10.20 ± 0.20	23.00 ± 0.30	27.00 ± 0.30	33.00 ± 0.34
Aspartate transaminase (AST) (U/L)	9.00 ± 0.20	30.00 ± 0.30	19.00 ± 0.30	28.00 ± 0.40
Alkaline phosphatase (ALP) (U/L)	111.90 ± 0.20	312.00 ± 0.50	281.00 ± 0.10	309.00 ± 0.40
Total protein (TP) (g/dL)	9.30 ± 0.20	8.30 ± 0.20	7.20 ± 0.20	9.70 ± 0.20
Albumin (ALB) (g/dL)	4.30 ± 0.80	4.90 ± 0.60	4.80± 0.30	3.20 ± 0.20
Creatinine (CRE) (mg/dL)	0.20 ± 0.10	0.30 ± 0.00	0.40 ± 0.10	0.30 ± 0.00
Urea (UR) (mg/dL)	12.90 ± 0.60	50.80 ± 0.60	65.00 ± 0.30	46.70 ± 0.70
Potassium (K) (mmol/L)	3.30 ± 0.30	2.70 ± 0.30	6.20 ± 0.40	3.90 ± 0.30
Sodium (Na) (mmol/L)	130.20 ± 0.40	136.00 ± 0.30	131.80± 0.40	138.20 ± 0.40
Chloride (Cl) (mmol/L)	97.00 ± 0.20	106.00 ± 0.30	105.10 ± 0.40	98.20 ± 0.30
Bicarbonate (HCO_3) (mmol/L)	18.10 ± 0.30	22.90 ± 0.30	18.80 ± 0.40	22.50 ± 0.40

Table 4.13: Effect of benzo (a) pyrene on biochemical indices in Wistar albino mice at different doses after 35 days' exposure

Parameters	Dosage			
	0 mg/kg	20 mg/kg	40 mg/kg	100 mg/kg
Total bilirubin (total bili) (mg/dL)	10.90 ± 0.40	12.00 ± 0.17	11.50 ± 0.13	13.90 ± 0.13
Direct bilirubin (direct bili) (mg/dL)	4.80 ± 0.20	6.40 ± 0.16	4.40 ± 0.16	5.30 ± 0.13
Alanine transaminase (ALT) (U/L)	10.20 ± 0.20	40.00 ± 0.16	41.00 ± 0.17	56.00 ± 0.16
Aspartate transaminase (AST) (U/L)	9.00 ± 0.20	53.00 ± 0.17	45.00 ± 0.16	60.80 ± 0.13
Alkaline phosphatase (ALP) (U/L)	111.90 ± 0.20	294.00 ± 0.16	297.00 ± 0.16	302.00 ± 0.17
Total protein (TP) (g/dL)	9.30 ± 0.20	8.60 ± 0.13	9.20 ± 0.16	11.60 ± 0.13
Albumin (ALB) (g/dL)	4.30 ± 0.80	5.10 ± 0.17	5.50 ± 0.13	7.40 ± 0.16
Creatinine (CRE) (mg/dL)	0.20 ± 0.10	0.30 ± 0.13	0.40 ± 0.16	0.40± 0.55
Urea (UR) (mg/dL)	12.90 ± 0.60	61.00 ± 0.13	64.80 ± 0.13	72.60 ± 0.13
Potassium (K) (mmol/L)	3.30 ± 0.30	4.10 ± 0.17	4.00 ± 0.17	3.80 ± 0.13
Sodium (Na) (mmol/L)	130.20 ± 0.40	133.90 ± 0.13	139.70 ± 0.13	141.00 ± 0.16
Chloride (Cl) (mmol/L)	97.00 ± 0.20	97.40 ± 0.16	101.10 ± 0.17	101.20 ± 0.16
Bicarbonate (HCO_3) (mmol/L)	18.10 ± 0.30	14.80 ± 0.13	20.30 ± 0.13	17.30 ± 0.13

4.3.4 Histopathological profile

The results of the haematoxylin - and eosin- stained sections of the lungs, stomachs, livers and kidneys of the aromatic hydrocarbons - treated Wistar albino mice are shown in Plates 4.1a – e, 4.2a – e, 4.3a – e and 4.4a – e.

From the lung results, there were normal tissue of the lung for control, congested lung with alveolar space containing red cells and dilated alveolar space for xylene at 1000 mg/kg, interstitium infiltrated by lymphocytes and plasma cells with associated vascular congestion as shown in the circular demarcation for anthracene at 100 mg/kg, vascular congested (VC) and oedematous lung for pyrene at 50 mg/kg and congested lung with terminal bronchus (TS) and alveolar space (AS) containing red cells for benzo (a) pyrene at 20 mg/kg.

From the stomach results, there were normal gastric mucosa tissue of the stomach for control, gastric fundic mucosa showing the acute and chronic inflammatory cells in the lamina propria for xylene at 1000 mg/kg, colonic mucosa showing tubular epithelial necrosis with luminal eosinophilic debris (ED) for anthracene at 100 mg/kg, glands with evidence of moderate stromal infiltration by chronic inflammatory cells due to chronic gastritis for pyrene at 50 mg/kg and stomach with mild gastritis for benzo (a) pyrene at 20 mg/kg.

From the liver results, there were normal liver tissues for control, evidences of fat liver disease - macrovesicular steatosis (MVS) with congested sinusoids (CS) and hepatocyte (HS) containing fat vacuoles (FV) for xylene at 1000 mg/kg, portion of the liver undergoing necrosis having a sharp contrast with macrovesicular steatosis (MVS)for anthracene at 100 mg/kg, fatty liver disease - macrovesicular steatosis (MVS) with central vein (CV) and hepatocyte (HS) containing fat vacuoles (FV) for pyrene at 50 mg/kg and portion of the liver

with macrovesicular steatosis (MVS) with hepatocyte (HS) containing fat vacuoles (FV) for benzo (a) pyrene at 20 mg/kg.

From the kidney results, there were normal tubules and glomeruli kidney tissue for control, tubular epithelial cells appearing eosinophilic with the cells at various stages of necrotic changes for xylene at 1000 mg/kg, renal tubular epithelial cells undergoing necrosis as evidenced by the presence of karyorrhexis and pyknosis of the nuclei with increased eosinophilia of the cytoplasmfor anthracene 100 mg/kg, presence of round basophilic bodies in the tubular lumens and luminal casts for pyrene at 50 mg/kg and portion of the kidney with luminal calcific castsfor benzo (a) pyrene at 20 mg/kg.

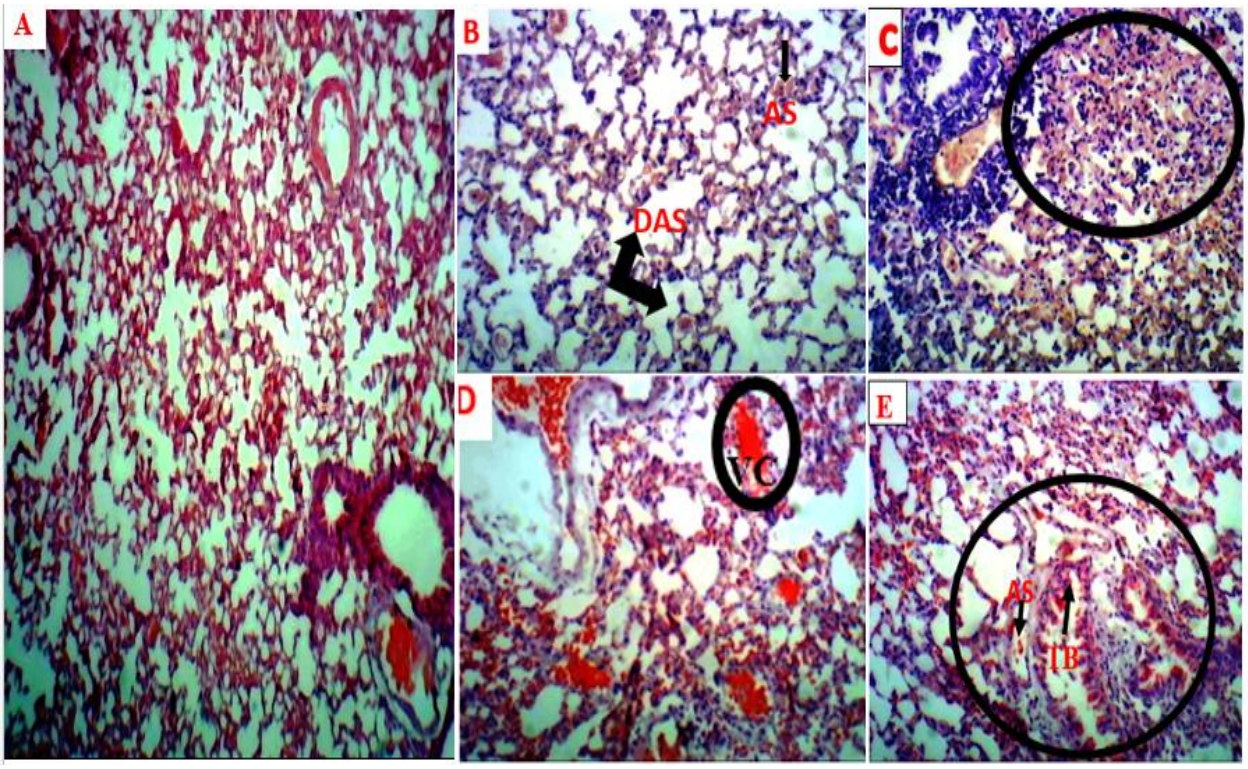


Plate 4.1a – e: Haematoxylin - and eosin- stained sections of the lungs of the aromatic hydrocarbon- treated Wistar albino mice. A. Normal tissue of the lung (X40) (control) B. Congested lung (AS- Alveolar space containing red cells, DAS- Dilated alveolar space) (X40) (xylene) C. Interstitium infiltrated by lymphocytes and plasma cells with associated vascular congestion as shown in the circular demarcation (X40) (anthracene) D. VC - Vascular congested and oedematous lung (X40) (pyrene) E. Congested lung (TB- Terminal bronchus, AS- Alveolar space containing red cells) (X40) (benzo (a) pyrene).

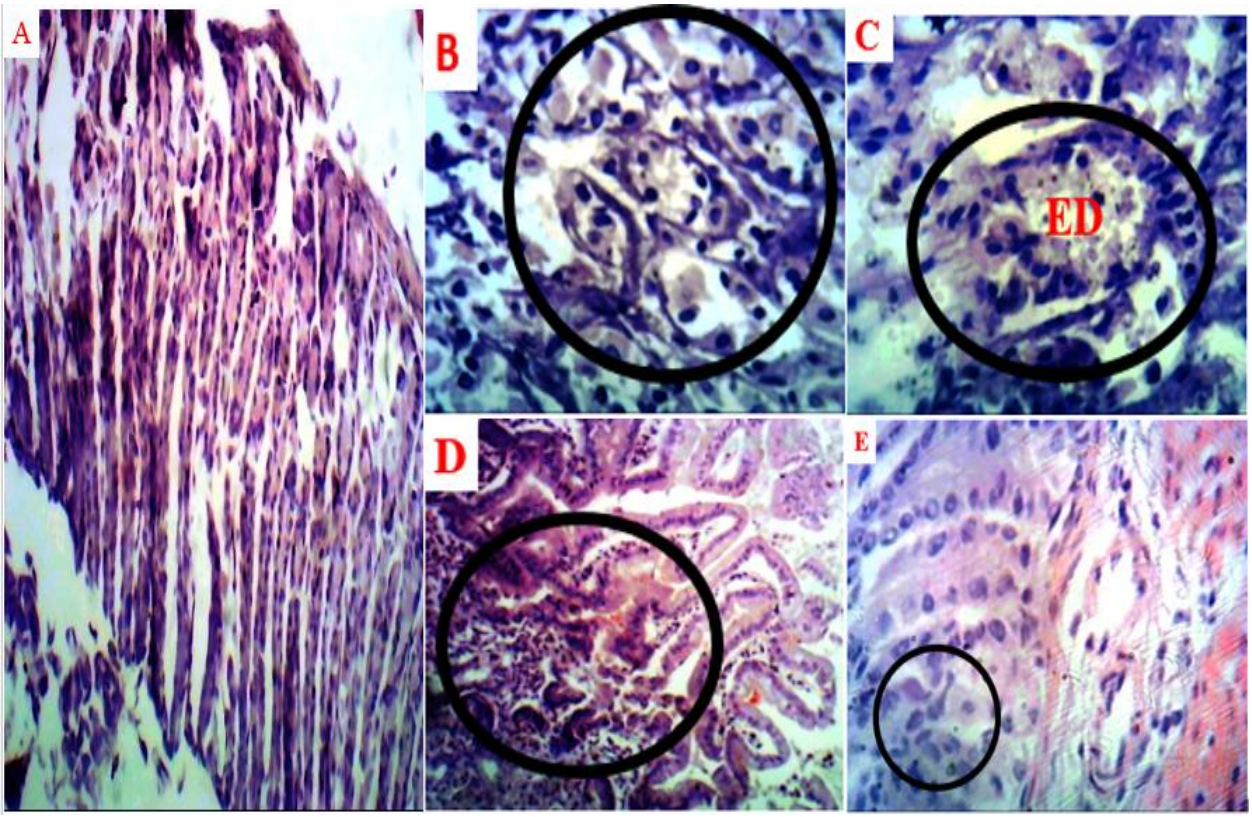


Plate 4.2a – e: Haematoxylin - and eosin- stained sections of the stomachs of the aromatic hydrocarbon- treated Wistar albino mice. A. Normal gastric mucosal(X40) (control) B. Gastric fundic mucosa showing the acute and chronic inflammatory cells in the lamina propria (X10) (xylene) C. Colonic mucosa showing tubular epithelial necrosis with luminal eosinophilic debris (ED) (X40) (anthracene) D. Glands with evidence of moderate stromal infiltration by chronic inflammatory cells due to chronic gastritis (X40) (pyrene) E. Stomach with mild gastritis(X40) (benzo (a) pyrene).

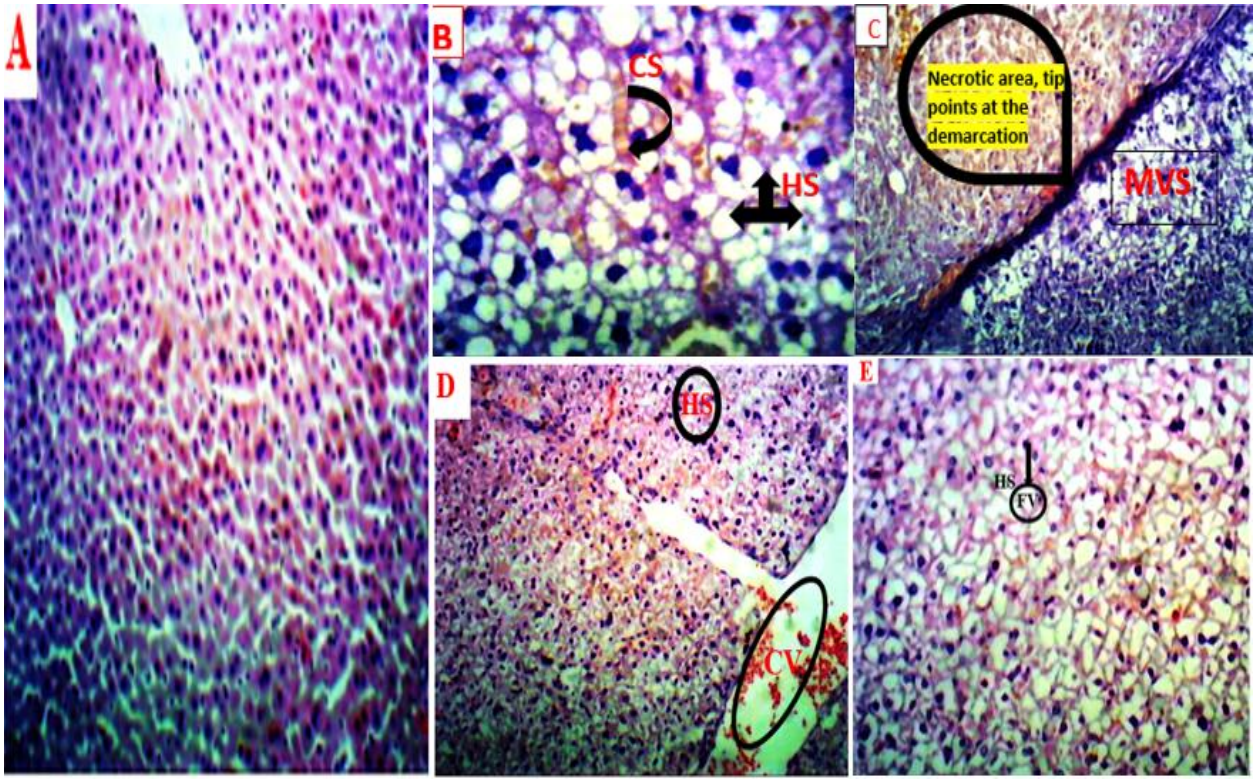


Plate 4.3a – e: Haematoxylin - and eosin- stained sections of the livers of the aromatic hydrocarbon - treated Wistar albino mice. A. Normal liver tissue (X10) (control) B. Fat liver disease - macrovesicular steatosis (CS- congested sinusoids, HS- hepatocyte containing fat vacuoles (X40) (xylene) C. Portion of the liver undergoing necrosis having a sharp contrast with macrovesicular steatosis (MVS)(X10) (anthracene) D. Fatty liver disease - macrovesicular steatosis (CV- central vein, HS- hepatocyte containing fat vacuoles) (X40) (pyrene) E. Portion of the liver with macrovesicular steatosis (HS- hepatocyte containing fat vacuoles - FV) (X40) (benzo (a) pyrene).

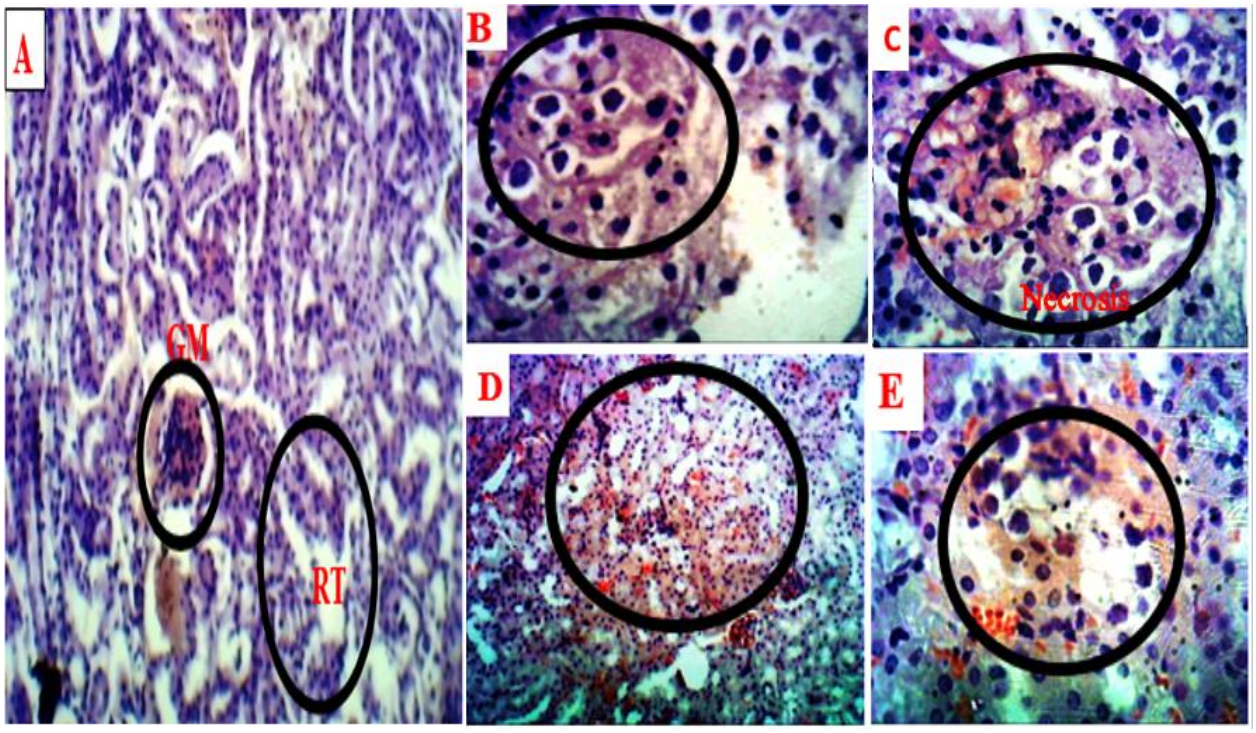


Plate 4.4a – e: Haematoxylin - and eosin- stained sections of the kidneys of the aromatic hydrocarbon - treated Wistar albino mice. A. Normal renal tubules and glomeruli kidney tissue(X40) (control) B. Tubular epithelial cells appearing eosinophilic with the cells at various stages of necrotic changes (X40) (xylene) C. Renal tubular epithelial cells undergoing necrosis as evidenced by the presence of karyorrhexis and pyknosis of the nuclei with increased eosinophilia of the cytoplasm(X40) (anthracene) D. Presence of round basophilic bodies in the tubular lumens and they are luminal casts (X4) (pyrene) E. Portion of the kidney with luminal calcific casts(X40) (benzo (a) pyrene).

4.4 Bacteriological Characteristics

4.4.1 Viable feature

The result of the mean total heterotrophic bacterial (THB) count of sediment and water samples from the three sampled locations is presented on Table 4.14. From the result, Nembe water had the highest mean THB log count of 18.95 ± 0.04 cfu/ml with highest percentage of occurrence of 32.40 ± 0.16 % while Onne sediment had the lowest mean THB log count of 5.34 ± 0.02 cfu/g with lowest percentage of occurrence of 2.90 ± 0.06 %, respectively. There were statistically significant counts detected using ordinary one-way ANOVA and Tukey's multiple comparison test among samples of the three locations ($P < 0.05$).

The result of the mean total hydrocarbon utilizing bacterial (THUB) count of sediment and water samples from the three sampled locations is presented on Table 4.15. From the result, Abonema sediment and water with xylene hydrocarbon had the highest mean THUB log count of 30.20 ± 0.12 cfu/g with highest percentage of occurrence of 17.30 ± 0.12 % and the lowest mean THUB log count of 04.35 ± 0.04 cfu/ml with lowest percentage of occurrence of 02.50 ± 0.04 %., respectivelyThere were statistically significant counts detected using ordinary one-way ANOVA and Tukey's multiple comparison test among samples of the three locations ($P < 0.05$) (Appendix VIp).

Table 4.14: Mean total heterotrophic bacterial (THB) count of sediment and water samples from the three sampled locations

Samples	Log count	Percentage (%)
Abonema water (CFU/ml)	13.60 ± 0.02	20.80 ± 0.16
Abonema sediment (CFU/g)	12.70 ± 0.02	18.90± 0.20
Nembe water (CFU/ml)	18.95 ± 0.04	32.40 ± 0.16
Nembe sediment (CFU/g)	11.50 ± 0.04	16.30 ± 0.16
Onne water (CFU/ml)	8.00 ± 0.16	8.70 ± 0.20
Onne sediment (CFU/g)	5.34 ± 0.02	2.90 ± 0.06

Values are mean ± standard deviation of triplicate determination.

Table 4.15: Mean total hydrocarbon utilizing bacterial (THUB) count of sediment and water samples from the three sampled locations

Sample	Log count	Percentage (%)
Abonema water + xylene (CFU/ml)	4.35 ± 0.04	2.50 ± 0.04
Abonema sediment + xylene (CFU/g)	30.20 ± 0.12	17.30 ± 0.12
Abonema water + anthracene (CFU/ml)	9.20 ± 0.08	5.30 ± 0.08
Abonema sediment + anthracene (CFU/g)	8.05 ± 0.01	4.60 ± 0.02
Abonema water + pyrene (CFU/ml)	4.42 ± 0.04	2.50 ± 0.04
Abonema sediment + pyrene (CFU/g)	9.45 ± 0.01	5.40 ± 0.02
Nembe water + xylene (CFU/ml)	12.35 ± 0.02	7.10 ± 0.06
Nembe sediment + xylene (CFU/g)	9.50 ± 0.01	5.50 ± 0.01
Nembe water + anthracene (CFU/ml)	10.50 ± 0.01	6.00 ± 0.12
Nembe sediment + anthracene (CFU/g)	6.09 ± 0.03	3.50 ± 0.02
Nembe water + pyrene (CFU/ml)	15.35 ± 0.15	8.80 ± 0.02
Nembe sediment + pyrene (CFU/g)	8.25 ± 0.02	4.70 ± 0.02
Onne water + xylene (CFU/ml)	7.60 ± 0.01	4.40 ± 0.02
Onne sediment + xylene (CFU/g)	7.70 ± 0.08	4.40 ± 0.08
Onne water + anthracene (CFU/ml)	5.20 ± 0.02	3.00 ± 0.06
Onne sediments + anthracene (CFU/g)	8.50 ± 0.02	4.90 ± 0.01
Onne water + pyrene (CFU/ml)	10.50 ± 0.02	6.00 ± 0.02
Onne sediment + pyrene (CFU/g)	6.95 ± 0.12	4.00 ± 0.12

Values are mean ± standard deviation of triplicate determination.

4.4.2 Screening and selection profile

The result of the aromatic hydrocarbon-degradability of marine bacterial isolates from Abonema sampled location is presented Appendix IIIa. From the result, thirteen (13) of the isolates show varying growth patterns with +++ as heavy growth, ++ as moderate growth and + as weak growth.

The result of the aromatic hydrocarbon-degradability of marine bacterial isolates from Nembe sampled location is presented Appendix IIIb. From the result, seventeen (17) of the isolates show varying growth patterns with +++ as heavy growth, ++ as moderate growth and + as weak growth.

The result of the aromatic hydrocarbon-degradability of marine bacterial isolates from Onne sampled location is presented Appendix IIIc. From the result, eighteen (18) of the isolates show varying growth patterns with +++ as heavy growth, ++ as moderate growth and + as weak growth.

The result of the growth performance ($OD6_{00}$ nm) of the aromatic hydrocarbon-degraders isolated from Abonema sampled location is presented in Table 4.16. From the result, 13 isolates were obtained with strains XYL2, ANT4 and PYR3 having the highest absorbance values of 0.952 ± 0.004, 0.775 ± 0.007 and 1.041± 0.008 on xylene, anthracene and pyrene hydrocarbons.

The result of the growth performance ($OD6_{00}$ nm) of the aromatic hydrocarbon-degraders isolated from Nembe sampled location is presented in Table 4.17. From the result, 17 isolates were obtained with strains XYL7, ANT1 and PYR5 having the highest absorbance values of 1.055 ± 0.002, 0.816 ± 0.007 and 0.933 ± 0.007 on xylene, anthracene and pyrene hydrocarbons.

The result of the growth performance ($OD6_{00}$ nm) of the aromatic hydrocarbon-degraders isolated from Onne sampled location is presented in Table 4.18. From the result, 18 isolates were obtained with strains XYL8, ANT6 and PYR9 having the highest absorbance values of 0.741 ± 0.007, 1.433 ± 0.013 and 0.871 ± 0.001 on xylene, anthracene and pyrene hydrocarbons.

Table 4.16: Growth performance of the aromatic hydrocarbondegraders isolated from Abonema sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
AB1	0.657 ± 0.008	0.657 ± 0.001	0.580 ± 0.003
ANT4*	0.676 ± 0.004	0.775 ± 0.007	0.822 ± 0.002
AB3	0.701 ± 0.021	0.467 ± 0.029	0.666 ± 0.003
AB4	0.715 ± 0.004	0.598 ± 0.024	0.841 ± 0.001
PYR3*	0.598 ± 0.005	0.511 ± 0.003	1.041 ± 0.008
AB6	0.641 ± 0.001	0.494 ± 0.002	0.653 ± 0.001
AB7	0.687 ± 0.004	0.690 ± 0.002	0.803 ± 0.016
AB8	0.618 ± 0.001	0.638 ± 0.007	0.782 ± 0.003
AB9	0.457 ± 0.002	0.475 ± 0.001	0.573 ± 0.004
XYL2*	0.952 ± 0.004	0.312 ± 0.002	0.838 ± 0.021
AB11	0.793 ± 0.014	0.495 ± 0.002	0.970 ± 0.003
AB12	0.647 ± 0.002	0.446 ± 0.001	0.621 ± 0.005
AB13	0.328 ± 0.001	0.415 ± 0.001	0.451 ± 0.001

KEY: * = Isolates with highest degradability; the values are mean ± Standard deviation of triplicate determination.

Table 4.17: Growth performance of the aromatic hydrocarbondegraders isolated from Nembe sampled location

Isolate	Optical density (OD_{600}nm)		
	Xylene	Anthracene	Pyrene
NW1	0.885 ± 0.003	0.236 ± 0.005	0.708 ± 0.008
PYR5*	0.710 ± 0.003	0.216 ± 0.005	0.933 ± 0.007
NW3	0.466 ± 0.007	0.201 ± 0.000	0.806 ± 0.004
NW4	0.893 ± 0.002	0.356 ± 0.008	0.827 ± 0.008
NW5	0.750 ± 0.004	0.132 ± 0.005	0.767 ± 0.008
NW6	0.644 ± 0.004	0.246 ± 0.004	0.724 ± 0.008
NW7	0.561 ± 0.003	0.193 ± 0.005	0.808 ± 0.001
NW8	0.628 ± 0.008	0.472 ± 0.001	0.826 ± 0.008
XYL7*	1.055 ± 0.002	0.588 ± 0.005	0.927 ± 0.001
NW10	0.809 ± 0.002	0.785 ± 0.002	0.881 ± 0.004
NW11	0.826 ± 0.001	0.444 ± 0.002	0.891 ± 0.001
NW12	0.625 ± 0.005	0.563 ± 0.001	0.728 ± 0.006
NW13	0.374 ± 0.008	0.775 ± 0.001	0.760 ± 0.001
NW14	0.701 ± 0.001	0.622 ± 0.003	0.788 ± 0.007
NW15	0.705 ± 0.008	0.529 ± 0.004	0.830 ± 0.002
NW16	0.769 ± 0.002	0.380 ± 0.001	0.822 ± 0.001
ANT1*	0.804 ± 0.003	0.816 ± 0.007	0.583 ± 0.001

KEY: * = Isolates with highest degradability; the values are mean ± Standard deviation of triplicate determination.

Table 4.18: Growth performance of the aromatic hydrocarbondegraders isolated from Onne sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
ON1	0.721 ± 0.001	0.884 ± 0.007	0.500 ± 0.001
ON2	0.204 ± 0.001	0.660 ± 0.011	0.454 ± 0.001
ON3	0.473 ± 0.003	0.476 ± 0.036	0.561 ± 0.013
ON4	0.207 ± 0.001	0.766 ± 0.001	0.565 ± 0.033
ON5	0.477 ± 0.002	0.457 ± 0.001	0.378 ± 0.005
ON6	0.409 ± 0.005	0.489 ± 0.100	0.562 ± 0.021
ON7	0.251 ± 0.003	0.428 ± 0.014	0.728 ± 0.001
ON8	0.111 ± 0.005	0.429 ± 0.014	0.425 ± 0.021
ON9	0.463 ± 0.008	0.357 ± 0.011	0.281 ± 0.006
PYR9*	0.106 ± 0.001	0.335 ± 0.001	0.871 ± 0.001
ON11	0.700 ± 0.001	0.901 ± 0.005	0.417 ± 0.002
ANT6*	0.511 ± 0.006	1.433 ± 0.013	0.568 ± 0.009
ON13	0.273 ± 0.002	0.386 ± 0.002	0.527 ± 0.001
ON14	0.278 ± 0.005	0.553 ± 0.022	0.684 ± 0.003
ON15	0.291 ± 0.003	0.748 ± 0.009	0.522 ± 0.010
ON16	0.662 ± 0.001	0.919 ± 0.002	0.494 ± 0.002
XYL8*	0.741 ± 0.007	0.510 ± 0.013	0.602 ± 0.004
ON18	0.354 ± 0.002	1.004 ± 0.001	0.478 ± 0.001

KEY: * = Isolates with highest degradability; the values are mean ± Standard deviation of triplicate determination.

4.4.3 Characteristics and identities of selected hydrocarbon utilizing bacterial isolates

4.4.3.1 Morphological and biochemical features of bacterial isolates

The results of aromatic hydrocarbon utilizing bacteria isolated on mineral basal agar plates are shown in Appendix IIId. From the results, many colonies with different colours, shapes and sizes were isolated on the enrichment medium agar plates after incubation for 14 days.

The result of the colonial descriptions of aromatic hydrocarbon degrading bacterial isolatesis presented in Table 4.19. From the result, most colonies were circular and irregular in shape, flat in elevation, undulate in margin, translucent in optic, smooth in texture, creamy in colour, 4 mm in size and glistering in surface description.

The result of the morphological and biochemical features of the aromatic hydrocarbon degrading bacterial isolates is presented in Table 4.20. From the result, most bacterial isolates were Gram negative in Gram reaction, rod shaped arranged in singles or pairs, negative to spore, indole, methyl red, Voges Proskauer, urease, gelatin, nitrate reduction, coagulase, hydrogen sulphide production, xylose, lactose, arabinose, maltose and casein hydrolysis tests while positive to catalase, motility, citrate, starch hydrolysis, mannitol, glucose, sucrose, saccharose and oxidase tests.

Table 4.19: Colonial descriptions of the aromatic hydrocarbon degrading bacterial isolates

Isolate	Colonialdescription							
	Shape	Elevation	Margin	Optics	Texture	Colour	Size	Surface
ANT1	Circular	Flat	Undulate	Translucent	Smooth	Creamy	4.0 mm	Dull
XYL2	Irregular	Raised	Erose	Translucent	Smooth	Creamy	3.0 mm	Dull
PYR3	Circular	Flat	Undulate	Translucent	Smooth	Creamy	2.0 mm	Glistering
ANT4	Circular	Flat	Erose	Translucent	Smooth	Creamy	5.2 mm	Glistering
PYR5	Circular	Flat	Undulate	Translucent	Smooth	Creamy	4.0 mm	Dull
ANT6	Rhizoid	Flat	Lobate	Translucent	Rough	Yellow	4.2 mm	Glistering
XYL7	Irregular	Flat	Undulate	Translucent	Rough	Red	4.0 mm	Glistering
XYL8	Irregular	Flat	Undulate	Translucent	Smooth	Creamy	4.2 mm	Glistering
PYR9	Irregular	Raised	Undulate	Translucent	Smooth	Creamy	4.0 mm	Glistering

KEY: ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

Table 4.20: Morphological and biochemical features of the aromatic hydrocarbon degrading bacterial isolates

Property	Isolate								
	ANT1	XYL2	PYR3	ANT4	PYR5	ANT6	XYL7	XYL8	PYR9
Gram reaction	_	_	_	_	_	_	_	_	+
Cellular morphology	Paired short rods	Singled long rods	Singled long rods	Singled short rods	Singled long rods	Single longer rods and long chains	Paired short rods	Single paired long Rods	Singled short rods
Spore test	_	_	_	_	_	_	_	_	+
Catalase	+	+	+	+	+	+	+	+	+
Indole	+	_	_	_	_	_	_	+	+
Motility	+	+	+	+	+	_	+	+	+
Methyl red	_	_	_	_	_	_	_	_	+
Voges-Proskauer	_	_	_	_	_	_	+	_	_
Citrate	_	+	_	+	+	+	+	_	+
Urease	+	_	_	_	_	_	_	+	+
Starch hydrolysis	+	+	_	+	_	+	_	+	+
Gelatin hydrolysis	_	_	_	_	_	_	+	_	_
NO_3 reduction	+	_	_	_	_	_	+	+	+
Coagulase test	+	_	_	_	_	_	+	+	_
H_2S production	+	_	_	_	_	+	_	+	+
Mannitol	+	_	_	_	_	+	+	+	+
Glucose	+	+	_	+	+	+	+	+	+
Xylose	_	_	_	_	_	_	+	_	+
Lactose	_	_	_	_	_	_	_	_	+
Sucrose	_	+	_	+	+	_	+	_	+
Arabinose	+	_	_	_	_	+	+	_	_
Maltose	_	_	+/_	_	_	_	_	_	+
Saccharose	+	+	_	+	+	_	+	+	+
Oxidase	_	+	+	+	+	+	+	_	+
Casein hydrolysis	_	_	_	_	_	_	+	_	_

KEY: NO_3 = Nitrate; H_2S = Hydrogen sulphide; = Negative result; + = Positive result; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

4.4.3.2 Molecular characteristics of the bacterial isolates

The result of the PCR - amplification of 16S rRNA genes of the aromatic - degrading bacterialgenomic DNA is shown in Plate 4.5. From the result, it was confirmed that the PCR products obtained using universal primer 16S-P1 PCR (27F 5′–3′: AGA GTT TGA TCC TGG CTC AG) and 16S-P2 PCR (1492R 5′–3′: ACG GCT ACC TTG TTA CGA CTT) have the molecular weight of 1 kbp visualized by UV fluorescence under agarose gel electrophoresis.

The results of the aligned gene sequences details of PCR amplified products respective to ANT1, XYL2, PYR3, ANT4, PYR5, ANT 6, XYL7, XYL8 and PYR 9 genes are presented in Appendices IVa - IVi. From the results, variable nucleotide sequences and lengths were obtained with sequencing primer 16S-PA SEQ (5′CTACGGGAGGCAGCAG3′) using automated DNA sequencer edit-view through autoassembler softwares.

The result of the percentage similarity and GenBank accession numbers of 16S rRNA sequences of the closest relative for the aromatic - degrading bacterial isolates is presented in Table 4.21. From the result, *Alcaligenes faecalis* was the most blasted organism with high similarity (98 - 99 %) followed by *Providencia* spp. (95 - 97 %), *Brevundimonas diminuta* (100 %), *Myroides odoratus* (90 %), *Serratia marcescens* (97 %) and *Bacillus cereus* (98 %) using National Centre for Biotechnology information (NCBI) BLAST software.

The result of neighbour-joining phylogenetic relationship among the 16S rRNA sequence of the aromatic - degrading bacterial isolates constructed by MEGA 7.0 is shown in Figure 4.12. From the result, it revealed that the nine bacterial isolates (KY171979, KY171984, KY171987, KY171980, KY171982, KY171981, KY171985, KY171986 and KY171983) show the same ancestry as they arise from the same node and hence they are evolutionary related.

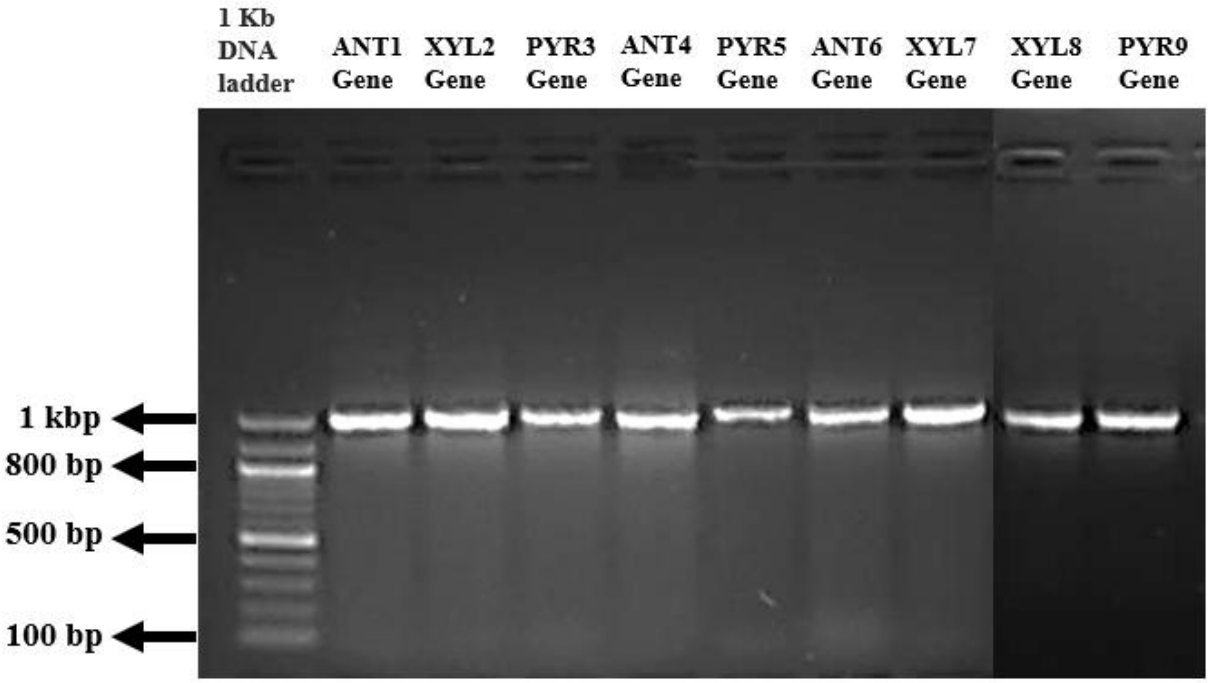


Plate 4.5: PCR - amplification of 16S rRNA genes of the aromatic degrading bacterial genomic DNA

KEY: ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

Isolate code	Closest relative in GenBank	Max score	Total score	Query coverage	E. value	Max identity	Accession Number
ANT1	*Providencia vermicola* strain MTCC 5578	544	544	93%	6e-151	95%	KY171979
XYL2	*Alcaligenes faecalis* strain MOR02	1559	4679	99%	0.0	99%	KY171984
PYR3	*Brevundimonas diminuta* strain zjs 01	1489	1489	97%	0.0	100%	KY171987
ANT4	*Alcaligenes faecalis* strain MOR02	1594	1594	99%	0.0	99%	KY171980

Table 4.21: Percentage similarity and GenBank accession numbers of 16S rRNA sequences of the closest relative for the aromatic - degrading bacterial isolates

PYR5	*Alcaligenes faecalis* strain MOR02	1537	4612	98%	0.0	98%	KY171982
ANT6	*Myroides odoratus* strain D25T	1194	1194	95%	0.0	90%	KY171981
XYL7	*Serratia marcescens* strain SM6	1476	1476	98%	0.0	97%	KY171985
XYL8	*Providencia* sp. strain X1	1491	1491	98%	0.0	97%	KY171986
PYR9	*Bacillus cereus* strain B4	1543	16940	98%	0.0	98%	KY171983

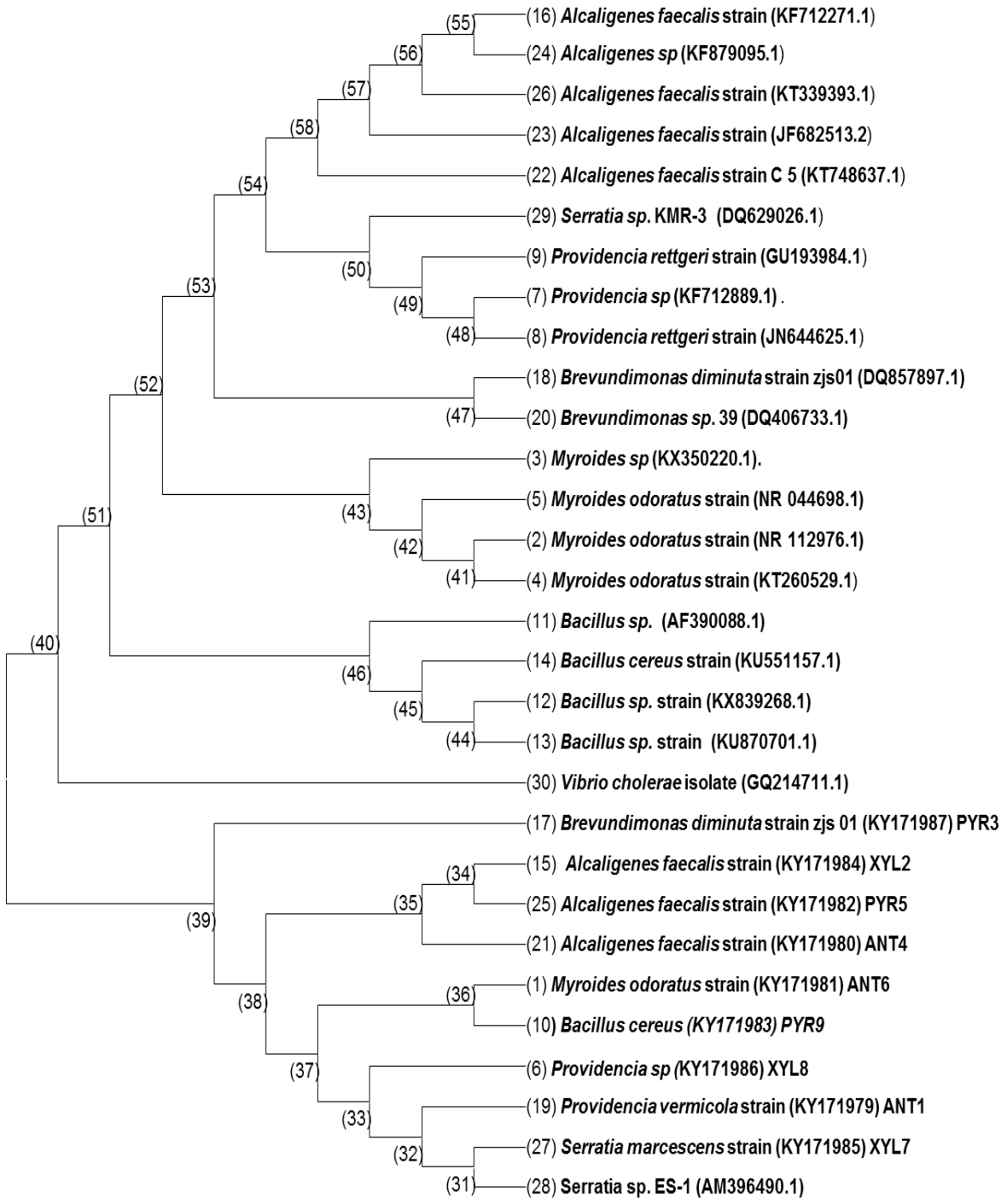


Figure 4.12: Neighbour-joining phylogenetic relationship among the 16S rRNA sequence of the aromatic - degrading bacterial isolates constructed by MEGA 7.0. Numbers at the nodes indicate bootstrap support (%) based on 1000 replicates. The sum of branch length = 0.93646865 using p-distance method involving 9 nucleotide sequences with total of 303 positions. GenBank accession numbers are given in parentheses.

4.4.4 Effect of aromatic hydrocarbon concentrations

The results of the growth of the selected aromatic hydrocarbon - degrading bacteria on different concentrations of xylene, anthracene and pyreneare shown in Figures 4.13, 4.14 and 4.15.

From the xylene result, the isolate *Alcaligenes faecalis* PYR5 had the least growth of 0.122 ± 0.003 (OD_{600nm}) observed at xylene of 300 ppm while *Providencia* sp. XYL8 had the best growth of 1.661 ± 0.297 recorded when exposed to 50 ppm of xylene for 5 days, respectively. From the anthracene result, the isolate *Providencia vermicola* ANT1 had the least and best growth of 0.048 ± 0.003 and 1.660 ± 0.020 (OD_{600nm}) when exposed to anthracene at 300 ppm and 50 ppm for 5 days, respectively.

From the pyrene result, the isolate *Providencia* sp. XYL8 had the least growth of 0.123 ± 0.001 (OD_{600nm}) observed at pyrene of 300 ppm while *Alcaligenes faecalis* XYL2 had the best growth of 1.330 ± 0.002 recorded when exposed to 50 ppm of pyrene for 5 days, respectively. There were extreme significant differences detected using ordinary one - way ANOVA and Dunnett's multiple comparison test among the treatment group of cell growth suspensions and the concentration of hydrocarbons ($P< 0.05$) with very strongly significant negative correlation ($P < 0.05$) (Appendices VIq and VIr).

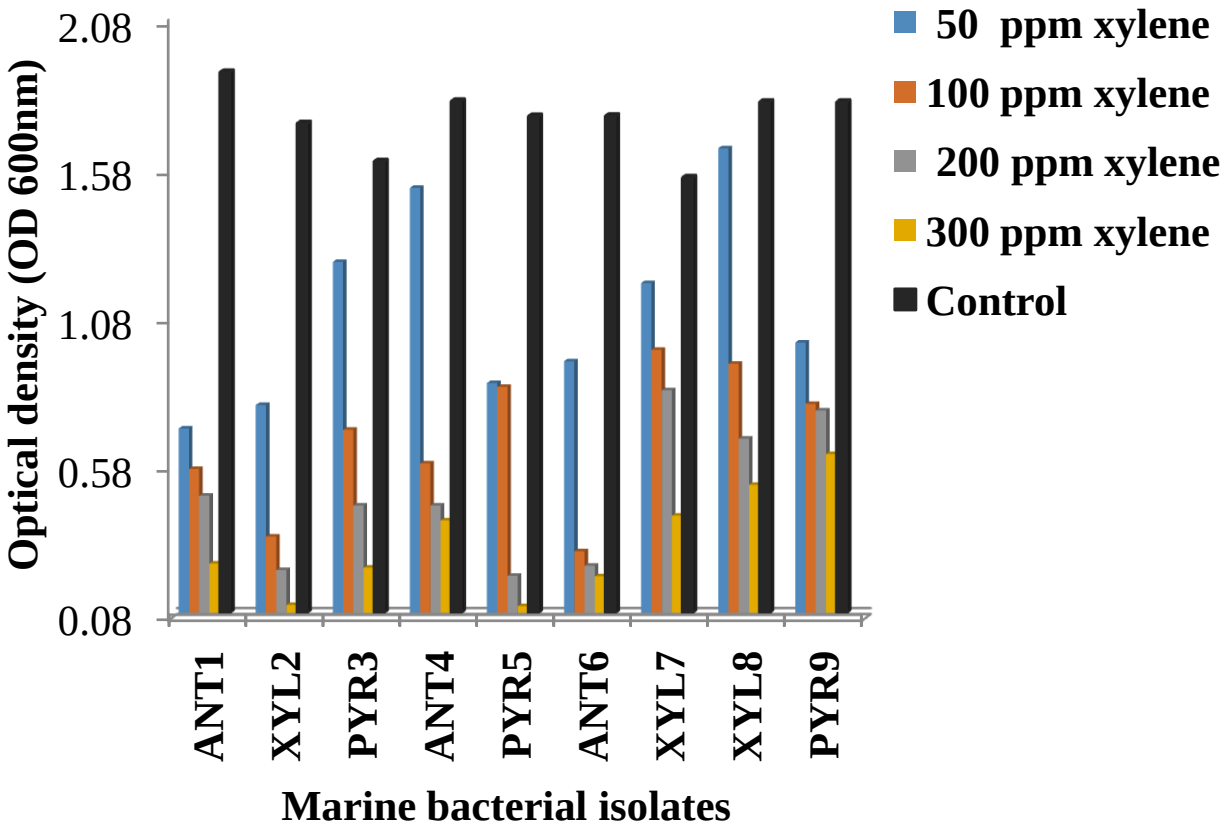


Figure 4.13: Growth of the selected aromatic hydrocarbon - degrading bacteria on different concentrations of xylene

KEY: PPM = Part Per Million; XYL = Xylene; ANT = Anthracene; PYR = Pyrene; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus;* $F(9, 40) = 5.75$; $P < 0.0001$; $R2 = 0.614 - 0.944$.

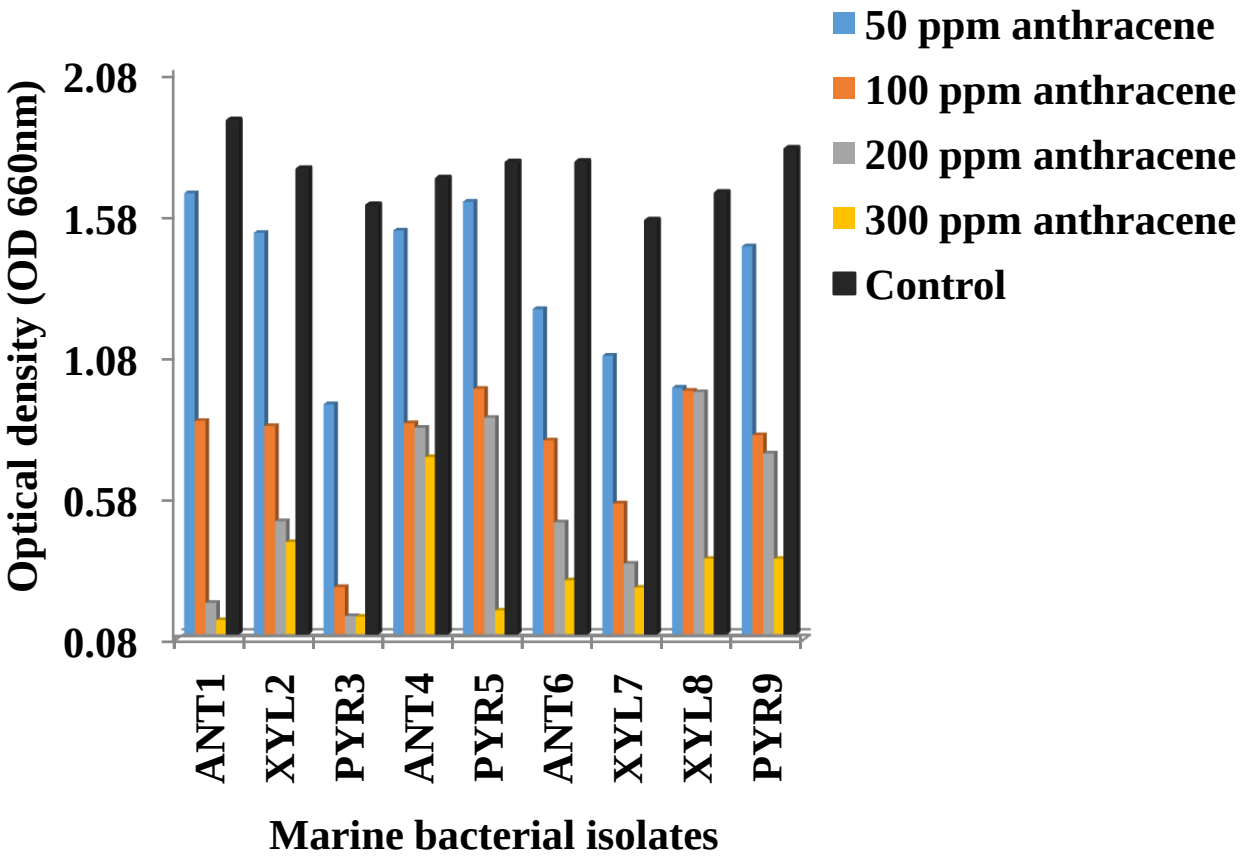


Figure 4.14: Growth of the selected aromatic hydrocarbon - degrading bacteria on different concentrations of anthracene

KEY: PPM = Part Per Million; XYL = Xylene; ANT = Anthracene; PYR = Pyrene; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus;* $F(9, 40) = 5.74$; $P < 0.0001$; $R2 = 0.676 - 0.932$.

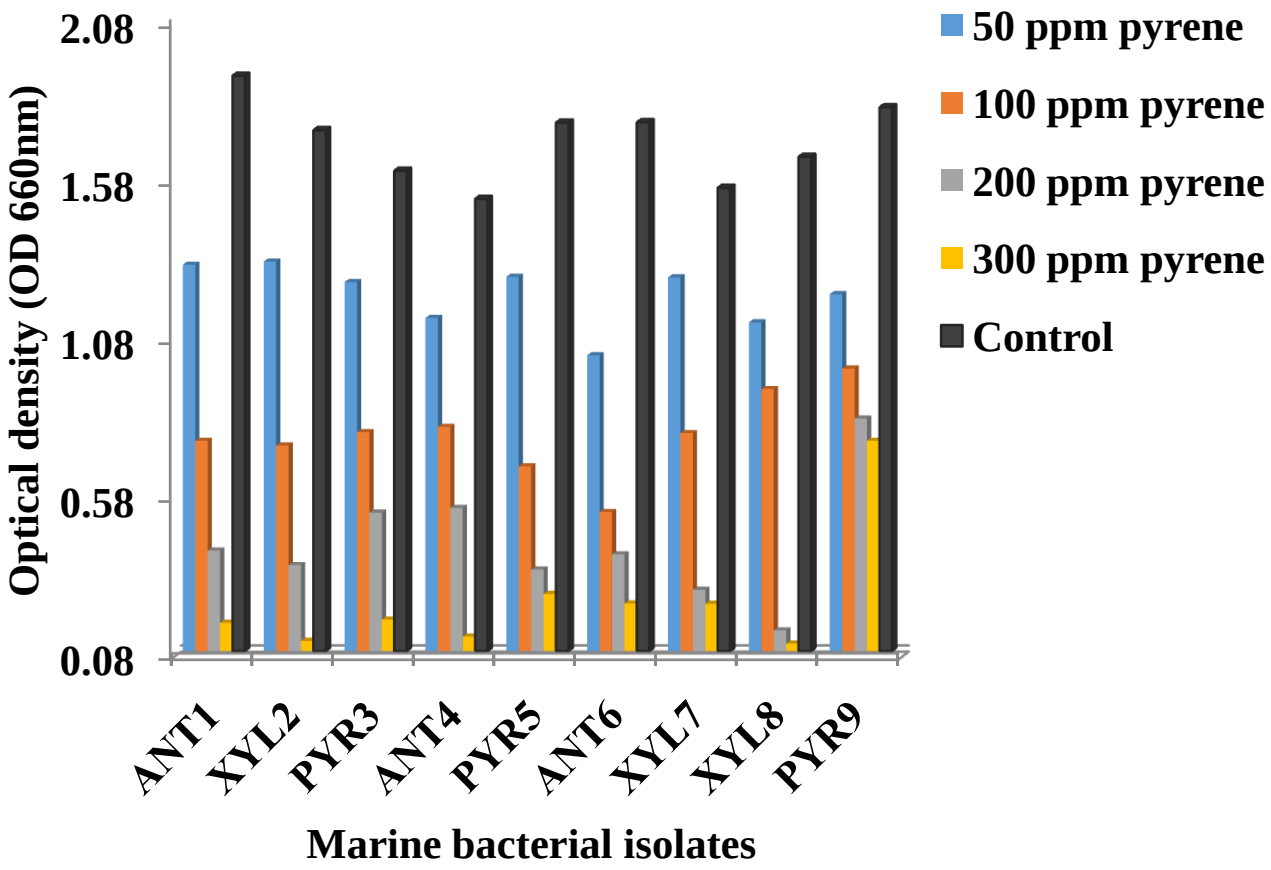


Figure 4.15: Growth of the selected aromatic hydrocarbon - degrading bacteria on different concentrations of pyrene

KEY: PPM = Part Per Million; XYL = Xylene; ANT = Anthracene; PYR = Pyrene; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus;* $F(9, 40) = 5.75$; $P < 0.0001$; $R2 = 0.739 - 0.961$.

4.4.5 Substrate specificity test

The result of the growth specific test of the marine bacterial isolates on crude oil and different hydrocarbons is presented on Table 4.22. From the result, the abilities of the isolates to degrade different hydrocarbons were found to vary. Isolate ANT1 had moderate/heavy growth on all the substrates while the other isolates had heavy, moderate, poor and no growth on the hydrocarbons substrates.

The result of the growth specific test of the marine bacterial isolates on different heavy metals is presented on Table 4.23. From the result, the abilities of the isolates to resist heavy metals were also found to vary. All the isolates had growth and resisted all metals except PYR3, ANT4 and ANT6 that were sensitive (–) to AsO_3 and CdO compounds. Isolates ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR 9 resisted and had heavy growths (+++) on $HgCl_2$, KFeCN, CdO, $PbSO_4$, $CuCl_2$, $K_2Cr_2O_7$, AsO_3, $MnCl_2$ and $ZnSO_4$ metallic compounds at 300 mg/l.

Table 4.22: Growth specific test of the marine bacterial isolates on crude oil and different hydrocarbons

Substrate	Isolate								
	ANT1	XYL2	PYR3	ANT4	PYR5	ANT6	XYL7	XYL8	PYR9
Crude oil	++	+++	+++	+++	++	+++	++	+	+++
Toluene	+++	+++	++	+++	−	−	−	−	−
Petrol	+++	−	++	++	+	−	−	+++	++
Petroleum ether	++	++	+	+++	−	++	+++	−	+
Engine oil	+++	++	+++	+++	++	+++	+	++	+++
Diesel	+	+	+++	−	+	+++	++	+++	+++
Hexane	+++	−	−	+++	+	+++	+++	+	+++
Kerosene	++	+	+++	++	+	+	+++	++	++
Catechol	+++	+	+	+	+	+++	+	++	+
Paraben	++	+	++	+++	++	++	+++	+++	+++

KEY: Growth was followed by measuring the increase of OD at 600 nm of the culture for 7 days; + + + Heavy growth: OD 600 nm > 0.2; + + Moderate growth: OD 600 nm 0.1 ≤ 0.2; + Poor growth: OD 600 nm 0.02 ≤ 0.1; - No growth: OD 600 nm < 0.02.ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

Table 4.23: Growth specific test of the marine bacterial isolates on different heavy metals

Isolate	Substrate (300 mg/l)								
	$CuCl_2$	AsO_3	$PbSO_4$	$HgCl_2$	$K_2Cr_2O_7$	KFeCN	CdO	$MnCl_2$	$ZnSO_4$
ANT1	++	+	+	+++	++	++	+	+	+
XYL2	++	+	+	++	++	+++	+	+	+
PYR3	+	–	+	+	+	+	+++	+	+
ANT4	+	-	+++	+	+	+	+	+	++
PYR5	+++	+	+	++	++	++	+	+	+
ANT6	++	–	+	++	+++	++	–	+	+
XYL7	+	+++	+	+	+	+	–	+	+
XYL8	+	+	+	+	+	+	+	+++	+
PYR9	+	+	++	+	+	+	+	+	+++

KEY: Growth was followed by measuring the increase of OD at 600 nm of the culture for 5 days; + + + Heavy growth: OD 600 nm > 0.2; + + Moderate growth: OD 600 nm 0.1 ≤ 0.2; + Poor growth: OD 600 nm 0.02 ≤ 0.1; - No growth: OD 600 nm < 0.02; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

4.4.6 Degradation profile

The results of the weight losses of xylene, anthracene and pyrene resulting from the growth of marine bacterial isolates are presented in Tables 4.24, 4.25 and 4.26.From the tables, *Serratia marcescens* XYL7 exhibited the highest capabilities to degrade the aromatic hydrocarbons with 99.50 ± 0.05 % and 60.00 ± 0.02 % reductions in weights of xylene and pyrene, respectively while *Alcaligenes faecalis* PYR5 degraded anthracene with 97.40 ± 0.01 % reduction in weight after 24 days' biodegradation study. There were extreme significant differences ($P < 0.05$) detected using ordinary two - way ANOVA and Dunnett's multiple comparison test among the treatment group of degraders and controls as well as very strongly significant positive correlation ($r = 0.897 – 0.996$) between incubation daysand the percentage degradation of hydrocarbons by the degraders (Appendix VIs).

Table 4.24: Weight loss of xylene result Days of incubation (Day) isolates

Isolate code	0	4	8	12	16	20	24
ANT1	0.248	0.026	0.024	0.021	0.018	0.016	0.015
		89.50 ± 0.02 %	90.30 ± 0.02 %	91.50 ± 0.01 %	92.70 ± 0.08 %	93.50 ± 0.01%	93.90 ± 0.06 %
XYL2	0.248	0.031	0.028	0.026	0.024	0.020	0.018
		87.50 ± 0.06 %	88.70 ± 0.01 %	89.50 ± 0.01 %	90.30 ± 0.02 %	91.90 ± 0.02 %	92.70 ± 0.06 %
PYR3	0.248	0.070	0.068	0.065	0.062	0.060	0.057
		71.80 ± 0.01 %	72.60 ± 0.03 %	73.80 ± 0.02 %	75.00 ± 0.08 %	75.80 ± 0.01 %	77.00 ± 0.01 %
ANT4	0.248	0.057	0.055	0.053	0.051	0.044	0.040
		77.00 ± 0.02 %	77.80 ± 0.01 %	78.60 ± 0.02 %	79.40 ± 0.06 %	82.20 ± 0.01 %	83.90 ± 0.02 %
PYR5	0.248	0.075	0.072	0.070	0.067	0.064	0.060
		69.80 ± 0.01 %	70.90 ± 0.07 %	71.70 ± 0.04 %	73.00 ± 0.08 %	74.10 ± 0.01%	75.80 ± 0.01 %
ANT6	0.248	0.061	0.059	0.057	0.054	0.052	0.050
		75.40 ± 0.02 %	76.20 ± 0.05 %	77.00 ± 0.06 %	78.00 ± 0.01 %	79.00 ± 0.08 %	79.80 ± 0.02 %
XYL7	0.248	0.019	0.018	0.017	0.015	0.010	0.001
		92.30 ± 0.02 %	92.70 ± 0.05 %	93.10 ± 0.02 %	93.90 ± 0.03 %	96.00 ± 0.02 %	99.50 ± 0.05 %
XYL8	0.248	0.046	0.044	0.042	0.040	0.038	0.035
		81.50 ± 0.04 %	82.30 ± 0.07 %	83.10 ± 0.31 %	83.90 ± 0.05 %	84.70 ± 0.06 %	85.90 ± 0.08 %
PYR9	0.248	0.057	0.053	0.050	0.048	0.046	0.043
		77.00 ± 0.02 %	78.60 ± 0.01 %	79.80 ± 0.05 %	80.70 ± 0.03 %	81.20 ± 0.01 %	83.90 ± 0.01 %
Control	0.248	0.246	0.243	0.241	0.240	0.237	0.235
		0.80 ± 0.02 %	2.00 ± 0.01 %	2.80 ± 0.05 %	3.20 ± 0.02 %	4.40 ± 0.05 %	5.20 ± 0.03 %

KEY: ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus;* Values are mean ± standard deviation of triplicate determination; $F(5,45) = 124$; $P < 0.001$.

Days of incubation (Day)

Table 4.25: Weight loss of anthracene resulting from the growth of marine bacterial isolates

Isolate	0	4	8	12	16	20	24
ANT1	0.386	0.089	0.088	0.086	0.085	0.085	0.084
		76.90 ± 0.05 %	77.20 ± 0.02 %	77.70 ± 0.01 %	77.90 ± 0.01 %	78.00 ± 0.01 %	78.20 ± 0.02 %
XYL2	0.386	0.098	0.090	0.084	0.075	0.060	0.031
		74.60 ± 0.08 %	76.70 ± 0.07 %	78.20 ± 0.08 %	80.60 ± 0.09 %	84.40 ± 0.02 %	91.20 ± 0.01 %
PYR3	0.386	0.098	0.096	0.094	0.093	0.092	0.091
		74.60 ± 0.02 %	75.10 ± 0.05 %	75.60 ± 0.06 %	75.90 ± 0.08 %	76.10 ± 0.02 %	76.40 ± 0.02 %
ANT4	0.386	0.105	0.104	0.103	0.102	0.101	0.100
		72.70 ± 0.05 %	73.00 ± 0.02 %	73.30 ± 0.06 %	73.60 ± 0.07 %	73.80 ± 0.03 %	74.00 ± 0.07 %
PYR5	0.386	0.060	0.050	0.040	0.030	0.020	0.010
		84.50 ± 0.03 %	87.00 ± 0.06 %	89.60 ± 0.07 %	92.20 ± 0.00 %	94.80 ± 0.02 %	97.40 ± 0.01 %
ANT6	0.386	0.070	0.066	0.054	0.050	0.043	0.046
		81.80 ± 0.02 %	82.90 ± 0.06 %	86.00 ± 0.09 %	87.00 ± 0.08 %	87.60 ± 0.04 %	88.00 0.06 %
XYL7	0.386	0.124	0.122	0.120	0.116	0.111	0.109
		67.90 ± 0.08 %	68.40 ± 0.08 %	68.90 ± 0.04 %	69.90 ± 0.01 %	71.20 ± 0.04 %	71.80 ± 0.07 %
XYL8	0.386	0.055	0.050	0.045	0.040	0.035	0.030
		85.80 ± 0.02 %	87.00 ± 0.06 %	88.30 ± 0.09 %	89.60 ± 0.01 %	90.90 ± 0.01 %	92.20 ± 0.01 %
PYR9	0.386	0.033	0.031	0.027	0.024	0.020	0.016
		91.50 ± 0.01 %	92.00 ± 0.04 %	93.00 ± 0.01 %	93.80 ± 0.08 %	94.80 ± 0.07 %	95.90 ± 0.01%
Control	0.386	0.383	0.380	0.376	0.374	0.370	0.368
		0.70 ± 0.023 %	1.50 ± 0.04 %	2.60 ± 0.06 %	3.10 ± 0.08 %	4.10 ± 0.07 %	4.60 ± 0.01 %

KEY: ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus;* Values are mean ± standard deviation of triplicate determination; F (5, 45) =13.05; P < 0.001.
Table 4.26: Weight loss of pyrene resulting from the growth of marine bacterial isolates

Days of incubation

Isolate	0	4	8	12	16	20	24
ANT1	0.117	0.078	0.075	0.073	0.071	0.069	0.068
		33.00 ± 0.08 %	35.90 ± 0.23 %	37.60 ± 0.07 %	39.30 ± 0.21 %	41.00 ± 0.06 %	41.90 ± 0.04 %
XYL2	0.117	0.098	0.086	0.083	0.081	0.079	0.077
		16.20 ± 0.03 %	26.50 ± 0.02 %	29.00 ± 0.06 %	30.80 ± 0.07 %	32.50 ± 0.08 %	34.20 ± 0.02 %
PYR3	0.117	0.103	0.100	0.099	0.097	0.094	0.090
		12.0 ± 0.07 %	14.5 ± 0.09 %	15.3 ± 0.03 %	17.0 ± 0.02 %	19.7 ± 0.08 %	23.0 ± 0.03 %
ANT4	0.117	0.097	0.090	0.089	0.086	0.084	0.080
		17.00 ± 0.05 %	23.00 ± 0.02 %	23.90 ± 0.01 %	26.50 ± 0.01 %	28.20 ± 0.05 %	31.60 ± 0.02 %
PYR5	0.117	0.067	0.064	0.062	0.060	0.057	0.053
		40.00 ± 0.06 %	45.30 ± 0.03 %	47.00 0.01 %	48.70 ± 0.04 %	51.30 ± 0.03 %	54.70 ± 0.03 %
ANT6	0.117	0.091	0.087	0.084	0.080	0.078	0.075
		22.00 ± 0.06 %	25.60 ± 0.06 %	28.00 ± 0.06 %	31.60 ± 0.09 %	33.30 ± 0.02 %	35.90 ± 0.02 %
XYL7	0.117	0.064	0.061	0.059	0.057	0.054	0.052
		45.30 ± 0.01 %	47.90 ± 0.05 %	49.60 ± 0.05 %	51.30 ± 0.09 %	53.80 ± 0.04 %	60.00 ± 0.02 %
XYL8	0.117	0.090	0.087	0.085	0.082	0.080	0.078
		23.00 ± 0.01 %	25.60 ± 0.05 %	27.40 ± 0.03 %	29.90 ± 0.01 %	31.60 ± 0.06 %	33.30 ± 0.06 %
PYR9	0.117	0.087	0.081	0.079	0.077	0.075	0.070
		25.60 ± 0.02 %	30.80 ± 0.07 %	32.50 ± 0.02 %	34.20 ± 0.03 %	35.90 ± 0.05 %	40.00 ± 0.05 %
Control	0.117	0.115	0.113	0.111	0.109	0.107	0.104
		1.70 ± 0.03 %	3.40 ± 0.02 %	5.10 ± 0.02 %	7.70 ± 0.06 %	8.60 ± 0.02 %	11.10 ± 0.06 %

KEY: ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus;* Values are mean ± standard deviation of triplicate determination; $F(5, 45) = 126$; $P < 0.001$.

4.4.7 Identification of metabolites formed/degradation products

The results of the thin layer chromatograms of xylene, anthracene and pyrene degradations in the control set-up and highest degraders are shown in Plates 4.6a – b, 4.7a – b and 4.8a – b. The results revealed that the the control set ups possess large high intensity spots while the highest degraders *Serratia marcescens* XYL7 and *Alcaligenes faecalis* PYR5 had small low intensity spots when visualized under UV illuminator at 235 nm with iodine spray.

The result of the retention factor of the aromatic hydrocarbons degraded by marine isolates as presented in Appendix Vwhich ranged from 0.27 ± 0.08 – 0.84 ± 0.01, 0.74 ± 0.08 – 1.00 ± 0.12 and 0.06 ± 0.03 – 1.00 ± 0.12 compared to controls which were 0.5 ± 0.01, 0.63 ± 0.02 and 0.38 ± 0.01 for xylene, anthracene and pyrene.

The results of Gas chromatograms of metabolites obtained during the incubation of marine isolates in the presence of xylene, anthracene and pyrene; GC retention data and electron impact mass spectral properties of metabolites formed from xylene, anthracene and pyrene utilization as well as proposed metabolic pathwayfor the degradation of xylene, anthracene and pyrene based on the degraded products identified using GC-MSby *Alcaligenes faecalis* strain XYL2, *Serratia marcescens* strain XYL7, *Providencia* sp. strain XYL8, *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain ANT4, *Myroides odoratus* strain ANT6, *Brevundimonas diminuta* strain PYR3, *Alcaligenes faecalis* strain PYR5 and *Bacillus cereus* strain PYR9 are presented in Tables 4.27, 4.28 and 4.29 and Figures 4.16, 4.17, 4.18, 4.19, 4.20 and 4.21. From the results, m – toluic acid, 4 - methoxylbenzenethiol, α – toluic acid, α – methylbenzylmethanol, catechol, propionic acid and acetaldehyde were the degradationproducts (metabolites) of xylene; paranaphthalene, 2 – ethyl – 9, 10 – anthraquinone, ethyl 6, 8 – difluoro – 4 -hydroxyquinoline – 3 –carboxylate, 1,2 - bis(trimethylsilyl) benzene, 1, 2, 3-trihydroxylbenzene, benzenecarboxylic acid and catechol were the degradation products(metabolites) of anthracene; while 9, 10 - dihydroanthracene, 4

– nitrophthalic acid, benzenecarboxylic acid, hydroxylmethylbenzene and catechol were the degradation products(metabolites) of pyrene.

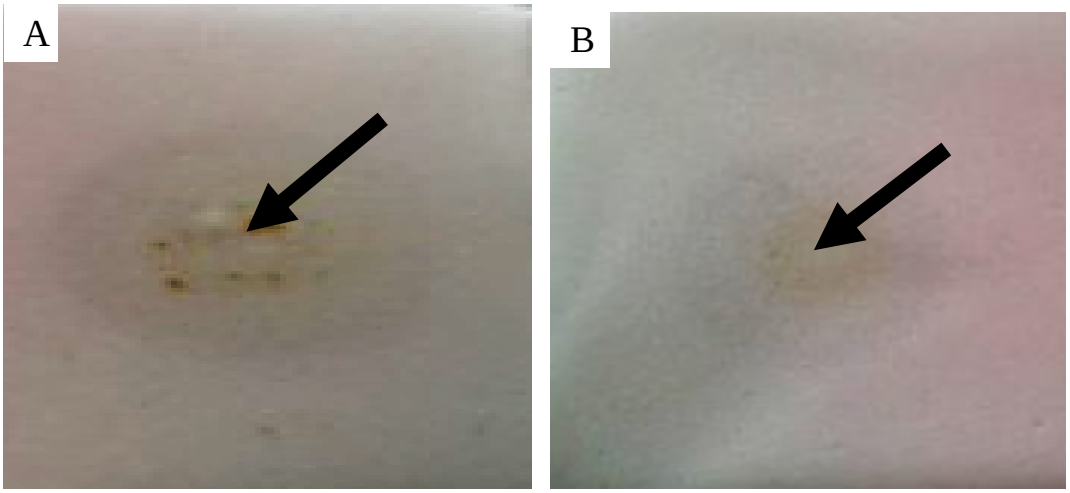


Plate 4.6a – b: Thin layer chromatograms of xylene degradation in the control set-up and highest degraders A. Thin layer chromatogram of xylene degradation in control sample with arrow showing large high intensity spot visualized under UV illuminator at 235 nm B. Thin layer chromatogram of *Serratia marcescens* XYL7 on xylene degradation with arrow showing small low intensity spot visualized under UV illuminator at 235 nm.

s

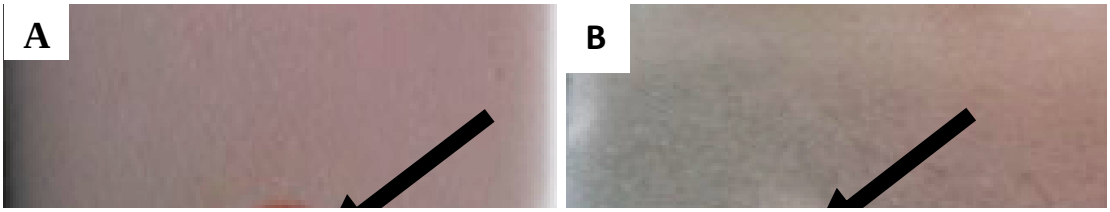


Plate 4.7a – b: Thin layer chromatograms of anthracene degradation in the control set-up and highest degraders A. Thin layer chromatogram of anthracene degradation in control sample with arrow showing large high intensity spot visualized under UV illu
ant
vis

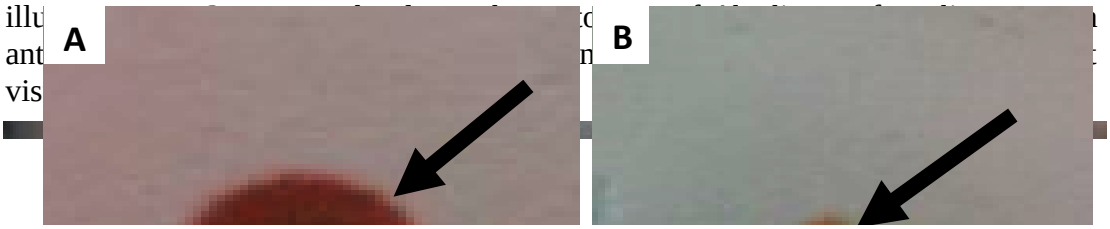


Plate 4.8a – b: Thin layer chromatograms of pyrene degradation in the control set-up and highest degraders A. Thin layer chromatogram of pyrene degradation in control sample with arrow showing large high intensity spot visualized under UV illuminator at 235 nm B. Thin layer chromatogram of *Serratia marcescens* XYL7 on anthracene degradation with arrow showing small low intensity spot visualized under UV illuminator at 235 nm.

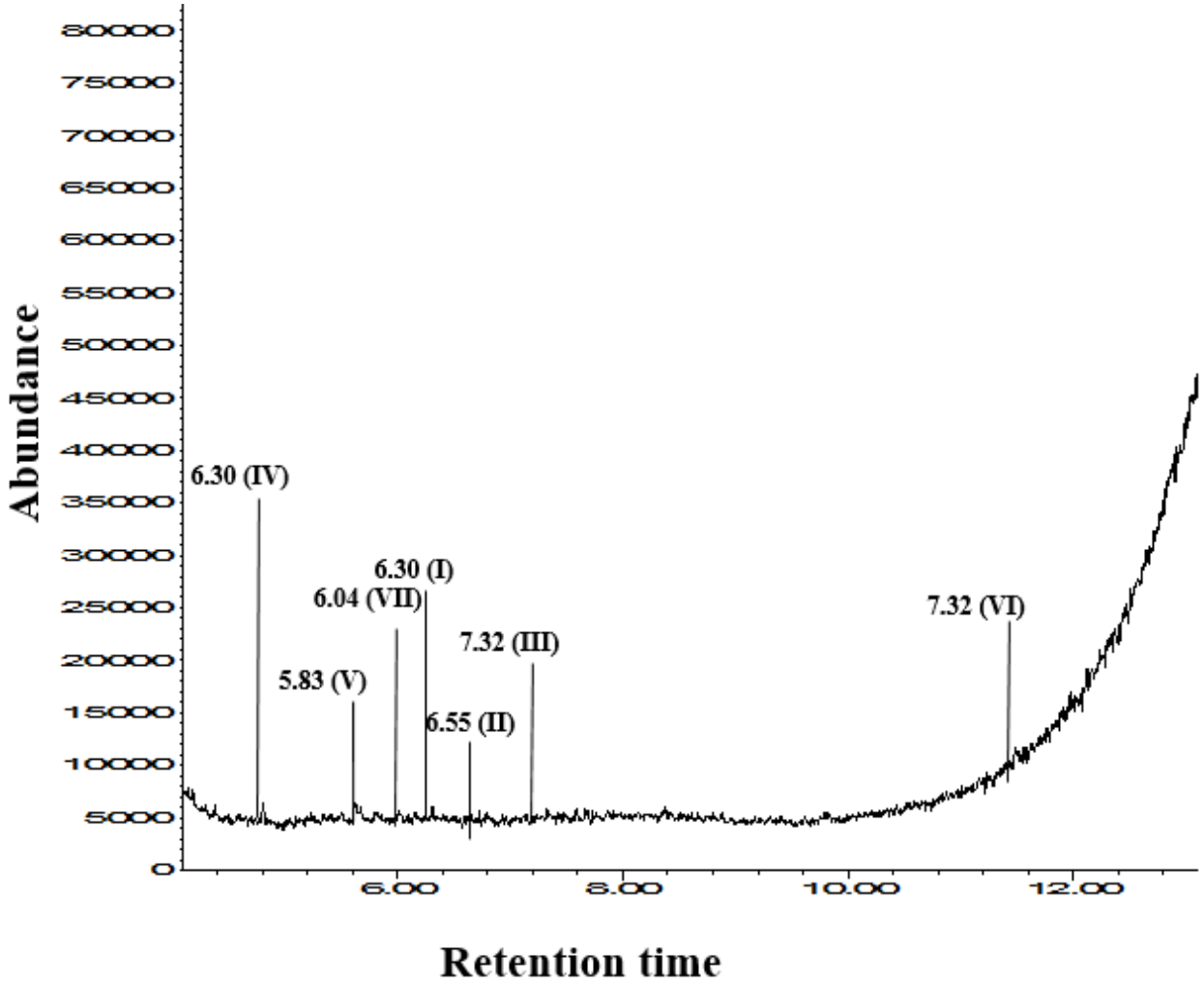


Figure 4.16: Gas chromatogram of metabolites obtained during the incubation of marine isolates in the presence of xylene. I. m – Toluic acid II. 4 – Methoxylbenzenethiol III. α – Toluic acid IV. α – Methylbenzylmethanol V. catechol VI. Propionic acid and VII. Acetaldehyde.

Metabolite	tR (min)	m/z ions (relative intensity/abundance)	Structural suggestion	Isolate code

i	6.30	136(100), 91(97), 119(57)	M – toluic acid	XYL7 and XYL8
ii	6.55	40(100), 125(78), 97(35)	4 - Methoxylbenzenethiol	XYL7 and XYL8
iii	7.32	91(100), 136(38), 92(19)	α – Toluic acid	XYL2, XYL7 and XYL8
iv	4.81	107(100), 122(86), 79(58)	α - Methylbenzylmethanol	XYL7 and XYL8
v	5.83	110 (100), 108 (15.55), 82 (18.37), 64(28.13),	Catechol	XYL2, XYL7 and XYL8
vi	11.56	74(100), 28(93), 45(90)	Propionic acid	XYL2 and XYL7
vii	6.04	29(100), 44(82), 43(48)	Acetaldehyde	XYL2, XYL7 and XYL8

Table 4.27: GC retention data and electron impact mass spectral properties of metabolites formed from xylene utilization by *Alcaligenes faecalis* strain XYL2, *Serratia marcescens* strain XYL7 and *Providencia* sp. strain XYL8

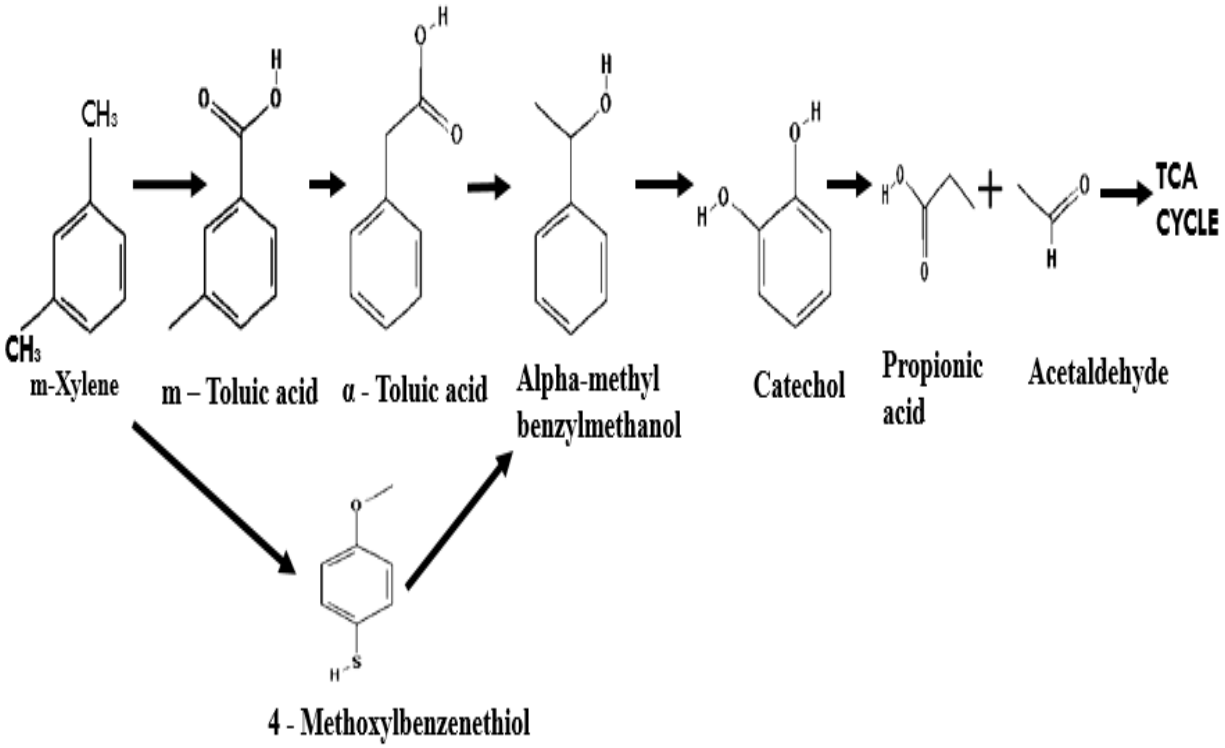


Figure 4.17: Proposed metabolic pathwayfor the degradation of the xylene by *Alcaligenes faecalis* strain XYL2, *Serratia marcescens* strain XYL7 and *Providencia* sp. strain XYL8 based on the degraded products identified using GC – MS

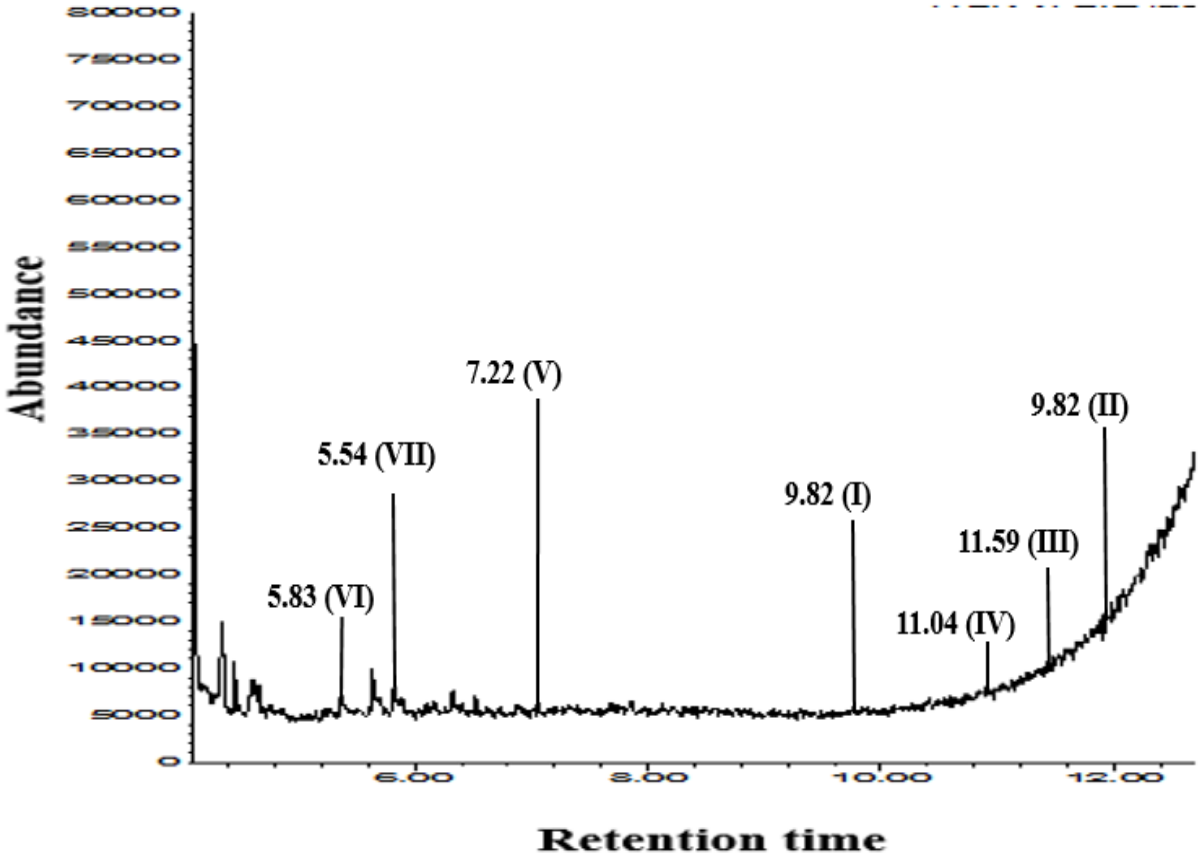


Figure 4.18: Gas chromatogram of metabolites obtained during the incubation of marine isolates in the presence of anthracene. I. Paranaphthalene, II. 2 – Ethyl – 9, 10 – anthraquinone III. Ethyl 6, 8 – difluoro – 4 -hydroxyquinoline – 3 –carboxylate IV. 1, 2 – Bis (trimethylsilyl) benzene V. 1, 2, 3 - Trihydroxylbenzene VI. Benzenecarboxylic acid and VII. Catechol.

Table 4.28: GC retention data and electron impact mass spectral properties of metabolites formed from anthracene utilization by *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain ANT4 and *Myroides odoratus* strain ANT6

Metabolites	tR (min)	m/z ions (relative intensity/abundance)	Structural suggestion	Isolate code
i	9.82	178(100), 179(15), 176(14)	Paranaphthalene	ANT4 and ANT6
ii	11.98	236(100), 221(30), 193(28)	2 – Ethyl – 9, 10 – anthraquinone	ANT1
iii	11.59	207(100), 253(25), 151(23)	Ethyl 6, 8 – difluoro – 4 -Hydroxyquinoline – 3– carboxylate	ANT1
iv	11.04	207(100), 73(27.5), 208 (22.5)	1,2 - Bis(trimethylsilyl) benzene	ANT4 and ANT6
v	7.22	126 (100), 52(47.5), 80(35)	1, 2, 3- trihydroxylbenzene	ANT1
vi	5.54	236(100), 221(30), 193(28)105(100), 122(84), 77(67.5)	Benzenecarboxylic acid	ANT1, ANT4 and ANT6
vii	5.83	110 (100), 108 (15.55), 82 (18.37), 64 (28.13),	Catechol	ANT1, ANT4 and ANT6

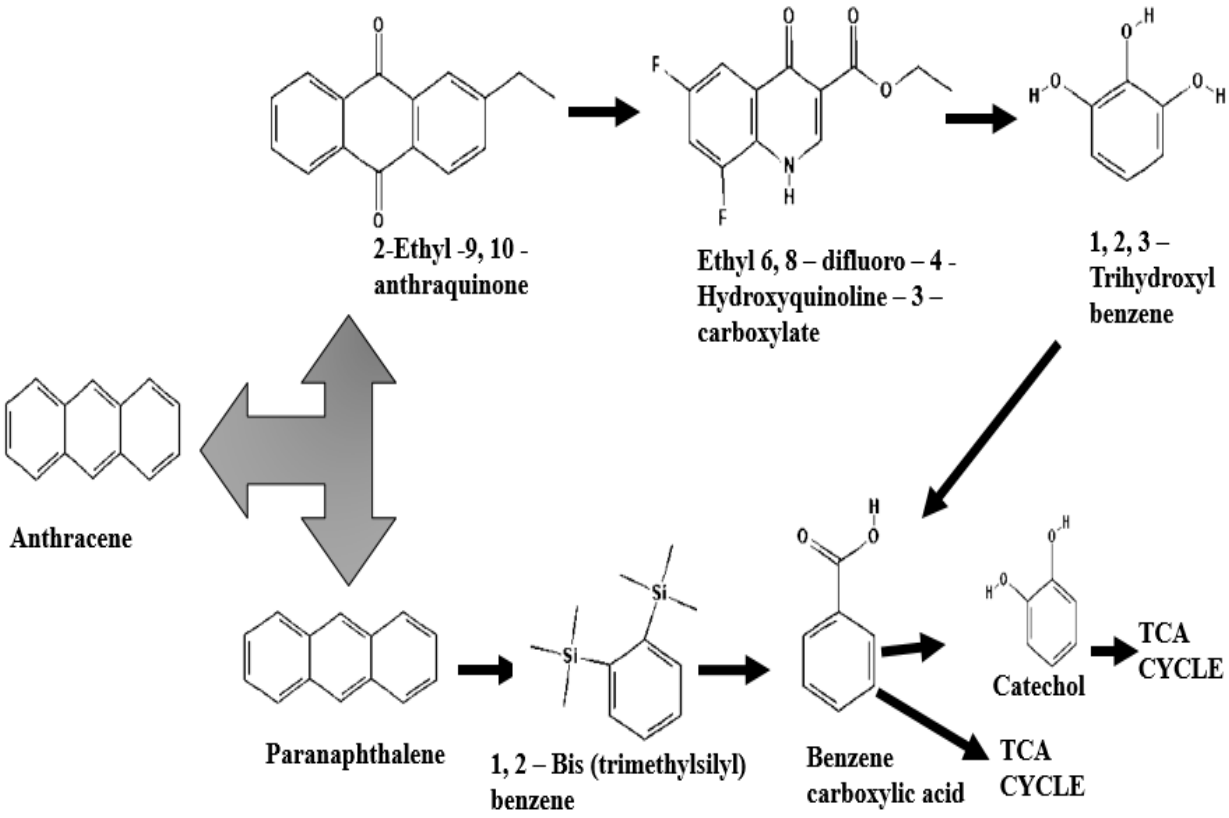


Figure 4.19: Proposed metabolic pathwayfor the degradation of the anthracene by *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain ANT4 and *Myroides odoratus* strain ANT6 based on the degraded products identified using GC-MS

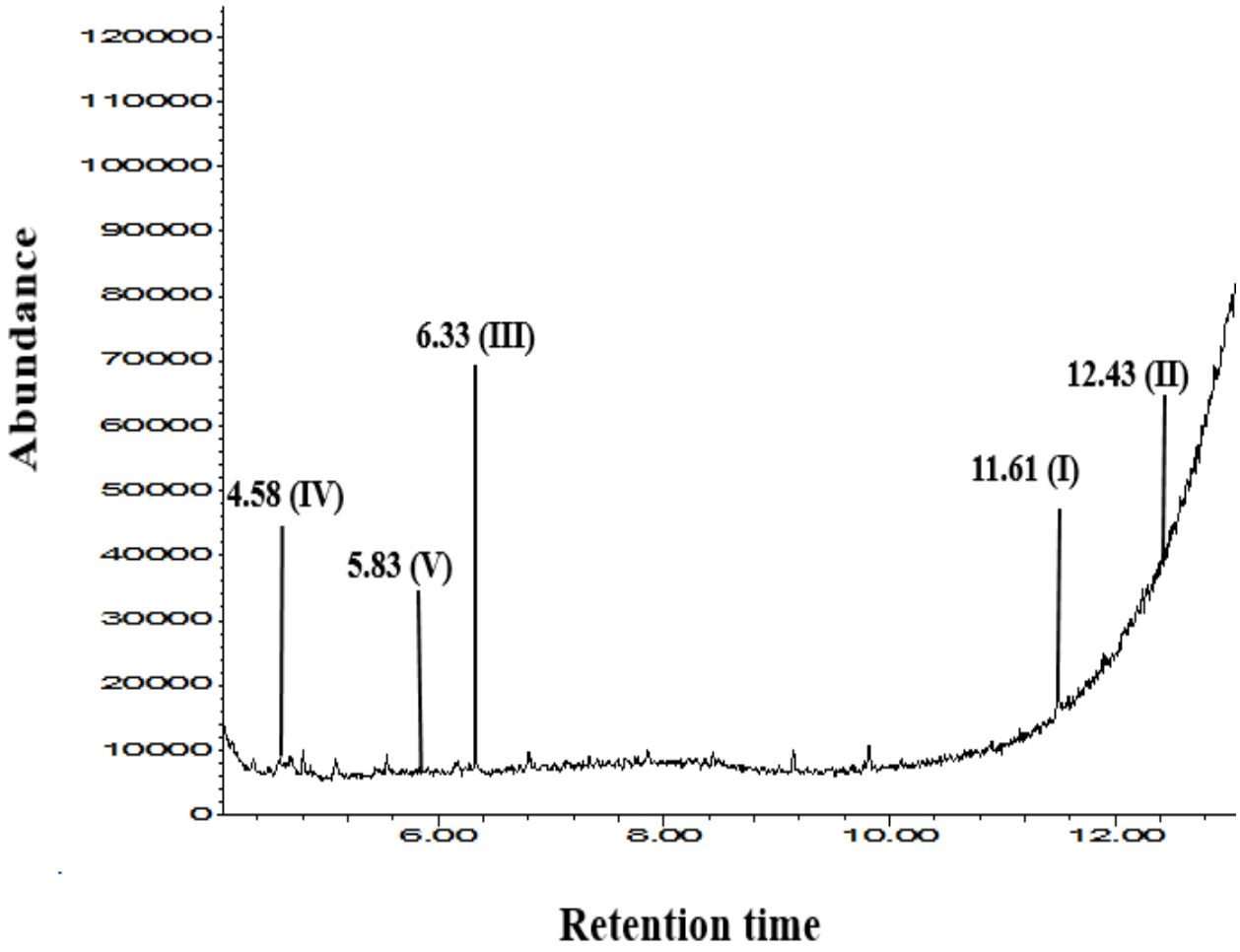
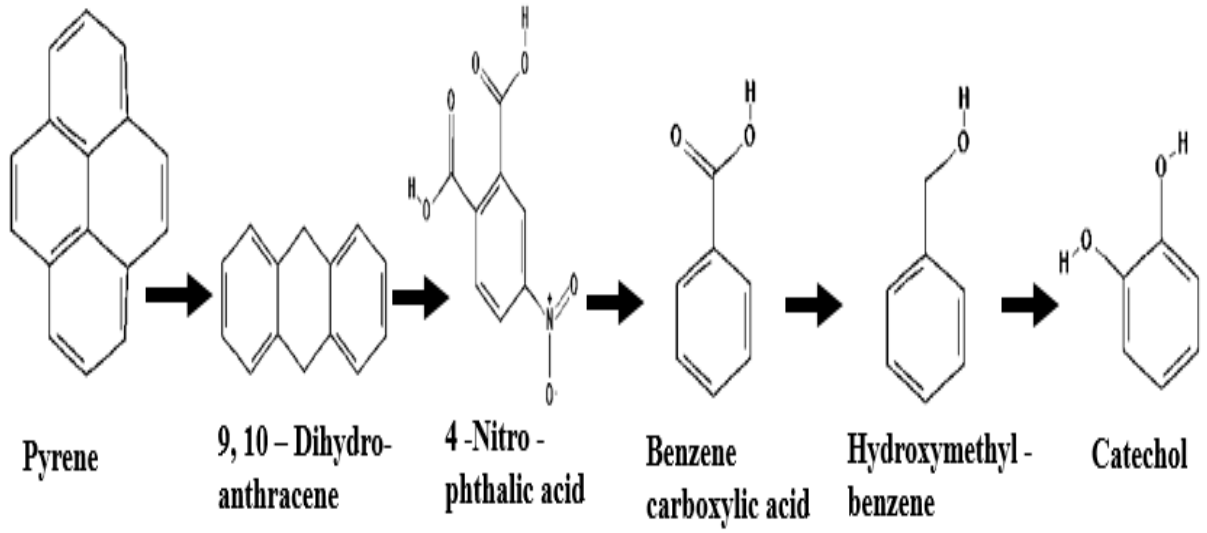


Figure 4.20: Gas chromatogram of metabolites obtained during the incubation of marine isolates in the presence of pyrene. I. 9, 10 - Dihydroanthracene II. 4 – Nitrophthalic acid III. Benzenecarboxylic acid IV. Hydroxylmethylbenzene V. Catechol.

Table 4.29: GC retention data and electron impact mass spectral properties of metabolites formed from pyrene utilization by *Brevundimonas diminuta* strain PYR3, *Alcaligenes faecalis* strain PYR5 and *Bacillus cereus* strain PYR9

Metabolite	tR (min)	m/z ions (relative intensity/abundance)	Structural suggestion	Isolate code
i	11.61	180(100), 179(94), 178(51.5)	9, 10 - Dihydroanthracene	PYR9
ii	12.43	149(100), 75(67), 103(65)	4 – Nitrophthalic acid	PYR3 and PYR5
iii	6.33	105(100), 122(84), 77(67.5)	Benzenecarboxylic acid	PYR3, PYR5 and PYR9
iv	4.58	79(100), 108(89), 107(71)	Hydroxylmethylbenzene	PYR3 and PYR5
v	5.83	110 (100), 108 (15.55), 82 (18.37), 64 (28.13),	Catechol	PYR3, PYR5 and PYR9



Brevundimonas diminuta strain PYR3, *Alcaligenes faecalis* strain PYR5 and *Bacillus cereus* strain PYR9

4.4.8 Detection of catabolic and surfactant genes by PCR analyses

The result of the catabolic and surfactant genes detected in some of the marine aromatic degrading bacterial isolates using specific primers for each gene is presented in Table 4.30 while the result of the PCR – amplification of primers specific for catabolic gene (*C23O*) and surfactant genes (*rhlB, SrfA3/LicA3*) of *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain XYL2, *Serratia marcescens* strain XYL7 and *Providencia* sp. strain XYL8 is shown in Plate 4.9. From the results, catabolic gene (*C23O*) was detected in only four (4) out of the nine marine aromatic degrading bacteria with name stated above while surfactant genes (*rhlB, SrfA3/LicA3*) were detected only in three (3) out of the nine marine aromatic degrading bacteria all of which had 881 base pairs sizes of PCR products of the catabolic and surfactant genes visualized by UV fluorescence under agarose gel electrophoresis, respectively.

Table 4.30: Catabolic and surfactant genes detected in some of the marine aromatic degrading bacterial isolates using specific primers for each gene

Isolate code	Bacterial name	Expected band (bp)	*C23O*	*rhlB*	*SrfA3/LicA3*
ANT1 Nembe	*Providencia vermicola*	881 bp	+	-	-
XYL2 Abonema	*Alcaligenes faecalis*	881 bp	+	+	-
PYR3 Abonema	*Brevundimonas diminuta*	-	-	-	-
ANT4 Abonema	*Alcaligenes faecalis*	-	-	-	-
PYR5 Nembe	*Alcaligenes faecalis*	-	-	-	-
ANT6 Onne	*Myroides odoratus*	-	-	-	-
XYL7 Nembe	*Serratia marcescens*	881 bp	+	+	+
XYL8 Onne	*Providencia* sp.	881 bp	+	+	+
PYR9 Onne	*Bacillus cereus*	-	-	-	-

KEY: + sign indicates the PCR product was detected and – sign means PCR product was not detected; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

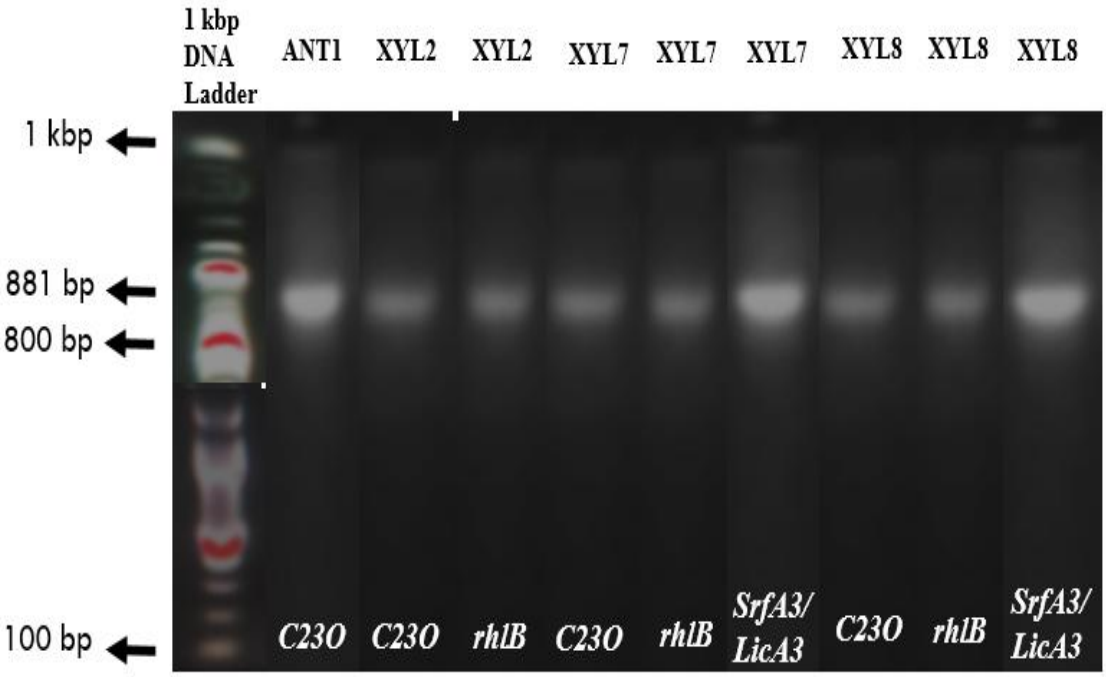


Plate 4.9: PCR – amplification of primers specific for catabolic gene (*C23O*) and surfactant genes (*rhlB, SrfA3/LicA3*) of *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain XYL2, *Serratia marcescens* strain XYL7 and *Providencia* sp. strain XYL8.

4.4.9 Plasmid analysis

The result of the aromatic hydrocarbon degradation before and after plasmid curing is presented in Table 4.31. From the result, all the nine marine bacterial strains that were successfully cured by sub –culturing in Nutrient broth lost the ability to degrade aromatic hydrocarbons namely xylene, anthracene and pyrene, respectively.

The result of the zones of inhibition of different antibiotics against the marine bacterial isolates is presented in Table 4.32. From the result, zones of inhibition were observed against tetracycline (10.00 ± 0.20 – 18.00 ± 0.10 mm), levofloxacin (14.00 ± 0.10 – 21.10 ± 0.00 mm), cefradoxil (11.30 ± 0.00 – 22.90 ± 0.20 mm), chloramphenicol (9.00 ± 0.10 – 17.30 ± 0.15 mm), erythromycin (8.00 ± 0.40 – 16.00 ± 0.00 mm) and azithromycin (9.50 ± 0.25 – 18.00 ± 0.30 mm) antibiotics while ampicillin (0.00 ± 0.00 mm) and amoxicillin (0.00 ± 0.00 mm) antibiotics showed no zone of inhibition.

The result of the electrophoretic separation profile of plasmid DNAs from cured and non-cured strains of the marine bacteria is shown in Plate 4.10. The result showed that seven of the nine selected isolates had multiple plasmids each except PYR3 and PYR5 that had single plasmid each.The two isolates were *Brevundimonas diminuta* PYR3 and *Alcaligenes faecalis*PYR5. The plasmids as revealed in Plate 4.10 below were of different sizes. The smallest is 200 bp while the largest was found to be above 1.2 kbp. The results also showed that the plasmids were partially cured hence the incomplete disappearance of all the bands after plasmid curing in the seven strains as shown in the Plate 4.10 except strains PYR3 and PYR5 respectively.

Isolate	Substrate	Before curing	After curing
ANT1 Nembe	Anthracene	+	_
XYL2 Abonema	Xylene	+	_
PYR3 Abonema	Pyrene	+	_
ANT4 Abonema	Anthracene	+	_
PYR5 Nembe	Pyrene	+	_
ANT6 Onne	Anthracene	+	_
XYL7 Nembe	Xylene	+	_
XYL8 Onne	Xylene	+	_
PYR9 Onne	Pyrene	+	_

Table 4.31: Aromatic hydrocarbon degradation before and after plasmid curing

Key: + = Ability to degrade; - = Lose ability to degrade; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

Table 4.32: Zones of inhibition (mm) of different antibiotics against the marine bacterial isolates

Isolate	Antibiotics							
	AMP	TE	LEV	CF	AMO	C	E	AZM
ANT1 Nembe	0.00 ± 0.00	11.00 ± 0.20	20.00 ± 0.10	22.00 ± 0.20	0.00 ± 0.00	10.00 ± 0.00	11.00 ± 0.20	13.00 ± 0.00
XYL2 Abonema	0.00 ± 0.00	11.70 ± 0.15	19.90 ± 0.21	18.80 ± 0.20	0.00 ± 0.00	10.70 ± 0.11	11.90 ± 0.21	14.70 ± 0.15
PYR3 Abonema	0.00 ± 0.00	13.80 ± 0.30	14.00 ± 0.00	19.80 ± 0.20	0.00 ± 0.00	10.00 ± 0.00	16.00 ± ± 0.00	12.50 ± 0.15
ANT4 Abonema	0.00 ± 0.00	10.00 ± 0.20	21.00 ± 0.00	20.00 ± 0.01	0.00 ± 0.00	9.00 ± 0.00	12.50 ± 0.00	12.00 ± 0.10
PYR5 Nembe	0.00 ± 0.00	11.40 ± 0.50	19.40 ± 0.10	22.90 ± 0.20	0.00 ± 0.00	5.00 ± 0.40	11.30 ± 0.20	10.90 ± 0.30
ANT6 Onne	0.00 ± 0.00	17.00 ± ± 0.00	20.0 ± 0.00	22.0 ± 0.20	0.00 ± 0.00	14.00 ± 0.00	14.00 ± 0.00	9.50 ± 0.25
XYL7 Nembe	0.00 ± 0.00	15.30 ± 0.20	13.50 ± 0.00	13.30 ± 0.00	0.00 ± 0.00	14.00 ± 0.10	14.00 ± 0.20	15.30 ± 0.15
XYL8 Onne	0.00 ± 0.00	18.00 ± 0.10	17.50 ± 0.20	16.90 ± 0.30	0.00 ± 0.00	17.30 ± 0.15	8.00 ± 0.40	18.00 ± 0.30
PYR9 Onne	0.00 ± 0.00	11.80 ± 0.40	21.10 ± 0.70	11.30 ± 0.30	0.00 ± 0.00	11.00 ± 0.00	12.10 ± 0.10	14.20 ± 0.20

KEY: AMP = Ampicillin; TE = Tetracycline; LEV = Levofloxacin; CF = Cefradoxil; AMO = Amoxicillin; AZM = Azithromycin; C = Chloramphenicol; E = Erythromycin; ANT1 - *Providencia vermicola*; XYL2 - *Alcaligenes faecalis;* PYR3 – *Brevundimonas diminuta;* ANT4 - *Alcaligenes faecalis;* PYR5 - *Alcaligenes faecalis*; ANT6 - *Myroides odoratus;* XYL7 - *Serratia marcescens*; XYL8 -*Providencia* sp. and PYR9 - *Bacillus cereus.*

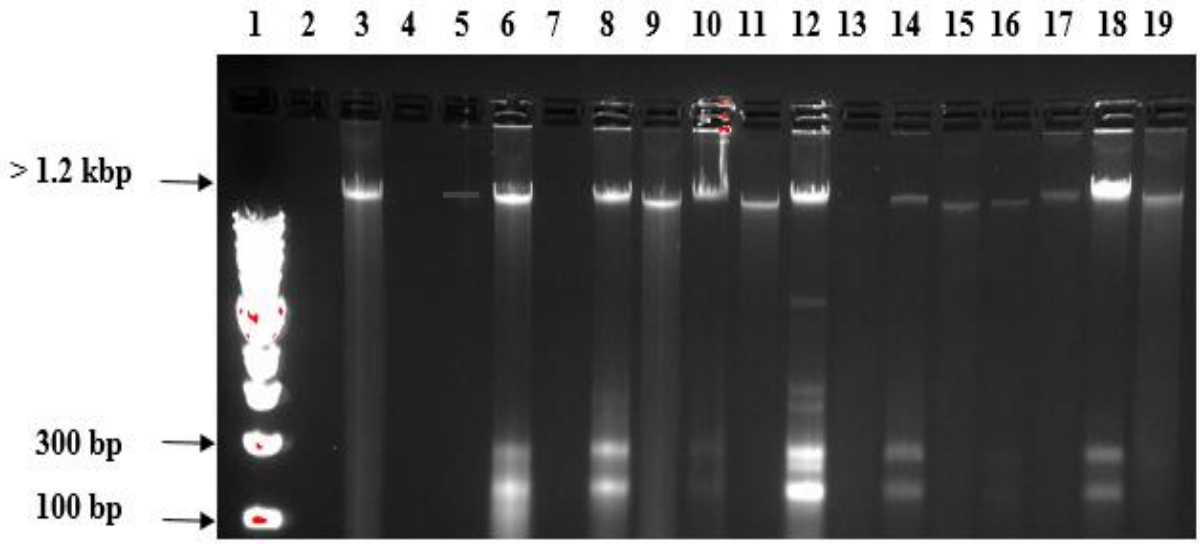


Plate 4.10: Electrophoretic separation profile of plasmid DNAs from cured and non-cured strains of the marine bacteria
KEY: 1 = 1 kbp DNA ladder; 2 and 3 = Cured and uncured strains of *Brevundimonas diminuta*PYR3; 4 and 5 = Cured and uncured strains of *Alcaligenes faecalis* PYR5; 6 and 7 = Uncured and cured strains of *Bacillus cereus* PYR9; 8 and 9 = Uncured and cured strains of *Alcaligenes faecalis*XYL2; 10 and 11 = Uncured and cured strains of *Serratia marcescens* XYL7; 12 and 13 = Uncured and cured strains of*Providencia* sp. XYL8; 14 and 15 = Uncured and cured strains of *Providencia vermicola* ANT1; 16 and 17 = Uncured and cured strains of *Alcaligenes faecalis*ANT4; 18 and 19 = Uncured and cured strains of *Myroides odoratus*ANT6.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Assessment of water or sediment samples using ecotoxicological assays should consist of a battery of tests, which includes a recommended minimum of three or more tests. The ideal set of tests should be representative of all the animals and plants, and all trophic levels of the ecosystem under investigation (Asker, 2011). The application of microbial bioremediation as a cost effective and eco-friendly treatment tool for the cleaning of certain oil -contaminated estuaries, shoreline, seas and oceans has received much attention since mechanical, physical and chemical treatments have limited effectiveness (Paniagua-Michel and Rosales, 2015).

In this study, toxicological evaluation and bioremediation potentials of bacteria isolated from contaminated marine environments of Rivers State, Nigeria was conducted to determine the toxicological effects of aromatic hydrocarbons and to explore the pattern of bacterial diversity, ecology and degradation potentials in polluted sediment/water samples inorder to obtain promising bacterial resources to be exploited for marine sites clean-up and the featuresof the three sampling sites are presented in Plates 3.1 – 3.3 and Appendices Ia – Ii.The result in Table 4.1 showed that there are more aromatic hydrocarbon fractions in sediment than water samples The results also showed that the detectable hydrocarbon values were above the WHO (2003) standard for PAHs (50 ng/l) in water. The reason may be due to the hydrophobic and insoluble nature of aromatic hydrocarbons to water molecules making them to adsorb on the surface of sediment substrata. Also, this result suggests that Nembe water side is more polluted than other sampled locations probably due to higher particle sizes,

higher total organic contents and the numerous anthropogenic activities that go on there, as a result of introducing and absorbing more aromatic hydrocabons. The result is similar to the work done by Gorleku *et al.* (2014), who reported that total mean concentrations of the PAHs in the sea water are generally less than the concentrations in sea sediments. Polycyclic aromatic hydrocarbons are non-polar, hydrophobic compounds which do not ionize. They have a relatively low solubility in water, but are highly lipophilic. Dissolved and colloidal organic fractions also enhance the solubility of PAHs which are incorporated into micelles. Due to their hydrophobic nature, PAHs entering the aquatic environment exhibit a high affinity for suspended particulates in the water column. As PAHs tend to adsorb to these particles, they are eventually settled out of the water column onto the bottom sediments. Thus, the PAH concentrations in water are usually quite low relative to the concentrations in the bottom sediments. Emoyan (2009) reported the concentration range of 0.2309 to 1.0468 mg l^{-1} for PAHs in surface water due to contamination from Kokorioil field in the Niger Delta. Fluorene was the dominant of the 16 PAH priority pollutants investigated. The source of water contamination was identified to be mainly petrogenic. The reported concentrations were higher than the CCME (2008) and the Netherland MPC guidelines. There were no statistically significant differences detected using ordinary one-way ANOVA and Tukey's multiple comparison test among the treatment group of aromatic hydrocarbons and the sampled locations ($P > 0.05$) (Appendix VIa).

Heavy metal pollution in the marine environment is determined by measuring its concentration in water, sediment and living organisms (Olusola and Festus, 2015). The result in Table 4.2 revealed that there are higher heavy metal contents in sediment than water samples and as such all metals except iron were within for water samples but higher for sediment samples in comparison to the Federal Ministry of Environment (FME, 2001) water

standards for aquatic life fresh (FWA) Nigeria and WHO (2008) maximum permissible recommended limits (MPL) and agreed with the finding of Olusola and Festus (2015). The studied elements are individually known to be mutagens and carcinogens. In other words, they are toxicants. The higher levels of these heavy metals in sediment of these coastal waters could be attributed to industrial and agricultural discharges, iron, steel and sewage materials from vessels and residential area and possible spills of petroleum products from fishing boats, speed boats and ships used as means of transportation over the years. Similar observations were made by Obiajunwa *et al*. (2002), who reported that the enrichment factors for Sr, Zn, Pb, Ba, and Fe were very high for every soil, sediment and solid waste samples in Niger Delta, Nigeria. The study concludes that there is significant relationship between heavy metal pollution and crudeoil production industry which suggests thatspillagehave occurred in the process of production. This is very harmful because the high contamination of heavy metal is very dangerous to both aquatic environment and human health. Hence, it is important to determine the concentrations of heavy metals in every difference level of environment in order to evaluate the possible risk of consumption (Ahmad *et al.*,2015). Owamah (2013) reported that the enrichment factors for Cd, Cr, Cu, Fe, Ni, and Pb were very high for water and sediment samples in Niger Delta, Nigeria. Olusola and Festus (2015) reported that the concentrations of Cd, Cu, Cr, Pb and Zn in the sediment samples of coastal waters of Ondo State were much below the probable effect concentration of sediment metal levels. Also, by comparing the concentrations of heavy metals analyzed in the water and sediment samples, it can be concluded that heavy metals are more highly accumulated in sediments than water confirming what has earlier been reported that sediments act as reservoir for all contaminants and dead organic matter (Bazzi, 2014; Olusola and Festus, 2015). There were no statistically significant differences detected using ordinary one-way ANOVA and Tukey's multiple

comparison test among the treatment group of heavy metals and the sampled locations ($P > 0.05$) (Appendix VIb).

The resultsin Tables4.3 – 4.4 revealed that Onne water and sediment had higher values of conductivity implying greater content of ions, currents and salts than other sampling locations although there can be natural variability such as temperature, tidal and seasonal flushing. Also, these results give surrogate valueslevels of salinities and total dissolved solids, (TDS). Hassanshahian *et al.* (2010)reported that the electrical conductivity of the Persian Gulf sediments was 6.5 (ds m^{-1}) in comparison with 8.4 (ds m^{-1}) value in the Caspian Sea sediments. Interestingly, the Persian Gulf is located in the South of Iran with warm weather and oil production area while the Caspian Sea is located in the North of Iran with rainy and temperate weather. Onne sediment and water samples exhibited lower pH values (06.72 ± 0.02 and 06.41 ± 0.02) followed by Abonema (07.47 ± 0.02 and 06.55 ± 0.02) and then Nembe (07.16 ± 0.02 and 07.31 ± 0.16) respectively. The samples were found to be acidic and neutral. The acidic pH value of the sandy loam sample could be interconnected with buildup of acidic metabolites and low mineral content of the soil (Ijah and Abioye, 2003). All the sediment samples had greater percentage of sand followed by clay and then silt and is similar to the results reported by Amer *et al.* (2015) that the sediments collected from the stations P, Q, and R located in El-Max district bay Mediterranian Sea Egypt are mainly composed of sand (85.82 – 95.62 %) while the sediment of station S displayed a different composition, containing approximately the same percentage of sand (39.41 %) and silt (34.39 %) and a higher proportion of clay (26.20 %) compared to the rest of the stations (0 – 5.49 %). Grain size measurements of superficial sediments revealed that Abonema and Nembe contained clayey loam whereas Onne contained sandy loam. The differences observed in the water content, porosity, bulk density, TOC, soil saturation and grain size are known to

influence the solubility of elements and nutrients in marine sediments, ultimately affecting the distribution of metals and other pollutants that preferentially bind to fine particles (Amer *et al*. 2015), determining as a consequence that the three sampled locations analyzed constitute different environmental niches. Both particle size and total organic content of sediments have been shown to be important factors in sediment PAH distribution, suggesting a particle size effect due to differences in adsorptive surface area (Gorleku *et al*., 2014). All the sampled locations showed high content of total nitrogen (TN), total phosphorus (TP) and exchangeable bases (potassium and calcium) in both sediment and water samples and the possible reasons for these occurrences may be due to pH and human activities observed along the study area which include agricultural landuse and farming operation, anthropogenic activities and industrialization. This report slightly contradicts the findings of Amer *et al*. (2015) who reported that all the stations showed total nitrogen content below 0.2 % w/v. Stations R and S showed a high content of total phosphorous with 0.83 and 0.59 ppm, respectively. Oyedele *et al*. (2008) reported that low pH (acidic) favours the abundance of exchangeable anions, but reduced cation, while high pH (basic) favours the abundance of exchangeable cations, but reduced anions in soils. Plants growing around the river in which the water has been discharged may experience excessive growth due to these nutrients while fish consumed from the river by humans will definitely have an adverse effect on them (Adenike, 2014). In the same vein, all the sampled locations COD and BOD were found to be higher than WHO (2008) standards except TDS, TSS and TS that were found below the maximum recommended limits. Ogunfowokan *et al*. (2005) observed significant elevation of water indices such as pH, BOD, nitrate, phosphate and TSS. It is well known that oxygen depletion in water bodies could cause fish death while increase in BOD signifies high load of organic matter. Also, organic matter decomposition in surface water produced inorganic nutrients such as ammonia, nitrate and phosphorus with resultant effects of eutrophication

and other serious ecological problems of such water body. There were no statistically significant differences detected using ordinary one-way ANOVA and Tukey's multiple comparison test among the treatment group of general parameters of sediment and water samples from the three sampled locations ($P > 0.05$) (Appendices VIc and VId).

The results in Appendices IIa - IIe showed that there were exponential growth patterns in the control test cultures ($\mu = 0.038h^{-1}$) while continuous inhibitions were observed at the varying test concentrations of the test samples. The control growth rate ($0.038\ h^{-1}$) is comparable to the minimal cell multiplication factor of 16 during a three-day test given in the Guideline (corresponding to a growth rate of $0.038\ h^{-1}$) which corresponds to EBPI (2016) guideline on validity criteria of the assay. The reasons for the significant growth in the control set ups could be due to the relatively high start cell density and absence of test substances in the test cultures while cell death could be the reason for the drastic reduction and decline in the test cultures with different concentrations of the test samples. Similarly, the result in Figure 4.1 revealed that there were drastic reductions in their respective specific growth rates leading to remarkable increase in the percentage inhibition values of all the test concentrations of the test samples. The fact that higher percentage of inhibitions were obtained showed that the test substances increasingly affected the growth at higher concentrations. Moreso, the result in Figure 4.2 showed that positive control ($K_2Cr_2O_7$) had the least toxicity while pyrene in Nembe sediment had the most toxicity to the microalgae. Generally, it could be deduced that the aromatic hydrocarbons and sediment samples possess acute aquatic toxicity (1 mg/l $< EC_{50} \leq$ 10 mg/l) than the individual test samples with very strong significant positive correlation between increasing test concentrations (0 -18 mg/l) and growth inhibitions to the marine microalga ($P < 0.05$) (Appendix VIe). There were no statistically significant differences detected using ordinary one-way ANOVA and Dunnett's multiple comparison

test among the treatment group of aromatic hydrocarbons, sediment samples and the positive control ($P > 0.05$) which indicates that inhibition effects among test samples were not much different from the control(Appendix VIe). This finding was similar to the work published by Oldersma *et al*. (2004), who reported that the statistical endpoints included in the model calculation, demonstrated a significant effect on the growth rate, and little or no effect on the growth of *Selenastrum capricornutum*(expressed as the area under the growth curve). The difference between the ErC_{50} and the EbC_{50} value therefore has no toxicological relevance. The ErC_{50} value of 4.4 mg l^{-1} is considered to provide the best expression of toxicity of *p* - xylene to algae. Aruoja*et al.*(2011) also found out that the EC_{50} values of *P. subcapitata* 72 h growth inhibition were experimentallydetermined for all 58 compounds. Despite the fact that the analyzed molecules were structurally similar, the EC_{50} values spanned two orders of magnitude ranging from 1.43 mg/l (3, 4, 5 - trichloroaniline) to 197 mg/l (phenol). The toxicity of the studied compounds was dependent on the type (chloro-, methyl-, ethyl-), number (mono-, di-, tri-) and position (ortho-, meta-, para-) of the substituents. As a rule, the higher the number of substituents the higher the toxicity. The chloro-substituted molecules were generally more toxic than alkyl-substituted ones. The results of the present study corroborate with the finding of these authors especially with the xylene hydrocarbon since it is almost structurally similar to the 58 aromatic compounds they studied.

The germination index (GI) gives an idea of the effect of contamination on both seed germination and root growth. The soils contaminated with aromatic hydrocarbons and sediment samples showed the lower GI values while individual test samples showed higher GI values. The most inhibitory effect was produced by pyrene in Onne sediment sample while the least inhibitory effect was produced by xylene in distilled water sample and the results in Appendices IIf, IIg and Figure 4.3revealed thatthe percentage of the inhibition of

root growth (IRG)on *Sinapsis alba*(mustard seeds)was higher in comparison with the inhibition of seed germination (ISG) and both of inhibition of seed germination and root growth of the test samples were much significantly different from the controls using ordinary one - way ANOVA and Dunnett's multiple comparison test among the treatment group of aromatic hydrocarbons, sediment samples and the control ($P < 0.05$) which indicates that inhibition effects among test samples were much different from the control(Appendix VIg). The reasons for the differences might be due to penetration and coating of the plant root tissues by the aromatic hydrocarbons and sediment samples thereby harming cell membranes, disruption and reduction of metabolic processes and reactions and subsequent killing of the embryo by direct acute toxicity or hazard. The result also revealed that over 80.00 ± 0.00 % of seeds in the negative control samples had a mean germination success with more than 20.00 ± 10.50 mm mean root length which corresponds to EBPI (2016) guideline on validity criteria of the assay, hence affirming the potential phytotoxic effect of aromatic hydrocarbon - contaminated sediments from the three sampled locations on tested *Sinapis alba* (Mustaed seeds). Similarly,Šestinová *et al*. (2012) reported that the percentage inhibition of seed germination (ISG)was 11.5 – 33.1 % and 12.5 – 33 % in the 1/2011-Hornad and 2/2011-Hnilec river samples respectively. The percentage of the inhibition of root growth (IRG)on *Sinapsis alba* seeds was higher in comparison with the inhibition of seed germination. It was 10.2 - 25.2 % and 18.6 - 42.5 % in the 1/2011-Hornád and 2/2011 - Hnilec sediments, respectively. Over 90 % of seeds in control samples germinated. Both the inhibitions of germination rate were not much different from the controls. Emami *et al.* (2014) reported that soils contaminated with petroleum showed the lowest GI values, and the inhibitory effect produced by petroleum were higher at the higher level of contamination, while addition of nitrogen fertilizer had a stimulatory effect on wheat seed germination.

The result in Appendices IIh, IIi and Figure 4.4 and 4.5 revealed that pyrene in Onne wastewater had the most hazardous effects while Abonema sediment had the least hazardous effectson the *Artemia franciscana* (brine shrimp)larvaeThe levels of toxicity class are drawn in accordance with International Regulations and National Legislative Program as followed: Highly toxic - LC_{50} / EC_{50}< 1mg/l; Toxic - 1mg/l<LC_{50}/ EC_{50} ≤ 10 mg/l; Harmful / hazardous for aquatic environment – 10 mg/l < LC_{50} / EC_{50}≤ 100 mg/l; Very low toxic, non-toxic - LC_{50} / EC_{50}> 100 mg/l (ASTM, 1992; GESAMP, 1997; CSP, 2016). These indicates that the aromatic hydrocarbon contaminated wastewater possess more potential lethal effects and moderate toxicities to *Artemia franciscana* (brine shrimp)larvae than the individual test sampleswith very strong significant positive correlation between increasing test concentrations (0 – 100 mg/l) and *Artemia* percentage mortality scores (% dead) of the marine crustacean (Appendix VIh). The result also revealed that less than 06.67 ± 0.00 % of mortality in the negative control samples was observed which corresponds to EBPI (2016) guideline on biological validity criteria (< 10%) of the assay. There were significant effects ($P < 0.05$) among the test samples but no significant differences detected using ordinary one-way ANOVA and Bonferroni's multiple comparisons test among the treatment group of aromatic hydrocarbons and the controls ($P > 0.05$) (Appendix VIi) which indicates that lethal effects among test samples were not much different from the controls. Khoshnood *et al.* (2017), reported that both TiO_2 and ZnO nanoparticles exhibited moderate toxicity to *Artemiafranciscana* larvae in 24h as compared with CuO, regardless of their size and concentrations. Rodd *et al.* (2014) reported that the mean mortality of untreated brine shrimp in their study was 1.2 %. After 24 h of benzene exposure, brine shrimp mortality is evident at an initial concentration of 75 mg/l. Mortality increases in an approximately linear fashion, with an LC_{50} of ~200 mg/l, until reaching ~100% mortality at a concentration of 500 mg/l benzene. The implications of dissolved aromatic hydrocarbons in petroleum/water systems is

that at high concentrations, inhalation of volatile or low – molecular weight hydrocarbons can cause irritation of the respiratory tract and central nervous system depression. Marine organisms including *Artemia* develop nonpolar narcosis that correlates with the octanol – water partition coefficient of the hydrocarbons.

The result in AppendicesIIj, IIk, and Figure 4.6 revealed that pyrene in Nembe water had the highest toxicity factor and xylene in distilled water had the lowest toxicity factor showing no significant weak positive correlation, respectively (Appendix VIk). This means that pyrene in Nembe water sample had very high significant acute toxicity potential than other samples. The trend of toxicity of the hydrocarbons is pyrene > anthracene > xylene showing that the increase in the number of benzene rings in aromatic hydrocarbons increases their level of toxicity as pyrenepossesses more benzene ring than anthracene and xylene, respectively. The toxicity factor of aromatic hydrocarbon - contaminated wastewaters were generally more than hydrocarbon contaminated distilled water. The reasons could be as a result of the interactions of these aromatic hydrocarbons,heavy metals and other organic and inorganic pollutants present in the sampled locations as they are generally known as toxicants. Adenike (2014) reported that the genotoxic effect of the tobacco waste effluent as validated from the various tests of his study can be a result of the interactions of heavy metals which can be more deleterious than the individual effects. All the samples had their coefficient of variation (CV %) to less than 25 thereby proving the biological validity criterion of the test and which corroborate the guideline values of EBPI (2016) that for Toxi-ChromoTest^{TM} quantitative results to be considered valid, the CV between the absorbance values of negative controls and sample replicates must be less than 25%. The result in Figure 4.7 revealed that xylene in distillled water had the least toxicity while pyrene in Onne water had the highest toxicityThe levels of toxicity class were also drawn in accordance with International Regulations and

National Legislative Program as followed: Highly toxic - LC_{50} / EC_{50}< 1mg/l; Toxic - 1mg/l <LC_{50}/ EC_{50} ≤ 10 mg/l; Harmful / hazardous for aquatic environment – 10 mg/l < LC_{50} / EC_{50}≤ 100 mg/l; Very low toxic, non-toxic - LC_{50} / EC_{50} > 100 mg/l (AST|M, 1992; GESAMP, 1997; CSP, 2016).Generally, it could be deduced that pyrene and anthracene are significantly (P <0.05) highly toxic aromatic hydrocarbons (EC_{50}< 1 mg /l) while xylene is a significantly (P < 0.05) toxic aromatic hydrocarbon (1 mg /l<EC_{50} ≤ 10 mg /l) compared to the positive control ($HgCl_2$) (EC_{50}< 1 mg/l)indicating that enzyme inhibition among test samples were much different from the positive control.There were statistically significant differences detected using ordinary one-way ANOVA and Dunnett's multiple comparison test among the means of the treatment group of aromatic hydrocarbons, wastewater and the control (P < 0.05) (Appendix VIj).These toxicity results (EC_{50}) are in line with other toxicity values for this type of pollutantas specified in the regulations above (AST|M, 1992; GESAMP, 1997; CSP, 2016). They are therefore consider being scientifically relevant in aquatic risk assessment.Previous research has demonstrated that Toxi – ChromoPad_solid phase test was sensitive for detecting bioavailable toxicants in sediments (Kwan and Dutka, 1995). Davoren *et al.* (2005) reported that based on the EC_{50}results for bioassay, Ballymacoda, Douglas and the East Wall sites estuarine sediments of Irish Coast containing PAHs and heavy metals were ranked as non -, moderately – and very toxic, respectively.Aruoja*et al.*(2011) also found out that the EC_{50} values of *V. fischeri*15 minutes' luminescence inhibition were experimentally determined for 28 aniline and 30 phenol compounds. Despite the fact that the analyzed molecules were structurally similar, the EC_{50} values spanned three orders of magnitude ranging from 0.37 mg/l(2, 3, 5-trichlorophenol) to 491 mg/l (aniline). The toxicity of the studied compounds was dependent on the type (chloro-, methyl-, ethyl -), number (mono-, di-, tri-) and position (ortho-, meta-, para-) of the substituents. As a rule, the higher the number of substituents the higher the toxicity. The

chloro-substituted molecules were generally more toxic than alkyl-substituted ones. The findings in their EC_{50} values were clearly higher than our results especially regarding xylene havingcomparable structural similarities with aniline and phenol compounds and the reason for these differences could be due to their organism's responses which differ from our mutant *E. coli* used in our study.

The result in Table 4.5 revealed that xylene 1000 mg/kg had the highest body weight before exposure and control without hydrocarbon had the highest body weight after exposure while the results in Figures4.8 – 4.11 showed that there were increases and decreases in mean weight of lung, stomach, liver and lung tissues. There were no statistically significant differences detected using paired T - test, repeated measures of one-way ANOVA and Dunnett's multiple comparison test among the time of exposure and means of body/organ weight of the treatment group of aromatic hydrocarbons and their controls ($P > 0.05$) (Appendices VIl – VIm).Xylene leads to reduced fetus weight and specially develops offspring size with skeletal deformities. So, it was concluded that xylene in huge amount lead to a foetus and parental toxic effects (Saillenfait *et al.*, 2003). Reduced body weight 20 and 42 days after birth has been shown in the offspring of CD-1 mice treated with BaP at 40 or 160 mg/kg body weight per day via oral gavage on days 7 through 16 of gestation, but not at 10 mg/kg bw per day (Health Canada, 2016).

The results in Tables 4.6 – 4.9 showed that there were varying decreases and increases in the haematological parameters of the Wistar albino miceat different doses of the aromatic hydrocarbons analyzed after 35 - days' study period. The control values were within the reference range but higher or lower than the treatment groups. The reasons for the variable changes in the haematological indices of the hydrocarbon - treated mice could be due to

apoptosis of red blood cell progenitors, pre-B cells and B lymphocytes induced by toxic effects of these aromatic hydrocarbons and their metabolites thereby leading to detrimental effect on bone marrow haematopoiesis. There were extremely significant differences detected using two - way ANOVA and Dunnett's multiple comparison test among the dose effect of the treatment group of aromatic hydrocarbons and their controls ($P < 0.05$) but not significant among the haematological indices ($P > 0.05$) (Appendix VIn). These findings corroborate with observation of Morcos *et al*. (2015) who reported that xylene injection caused a significant decrease in RBCs, platelets and haemoglobin levels. The decrease was correlated to time for the RBCs, and haemoglobin, while the count of WBCs increased after 0.5 and 0.62 mg/Kg doses, then declined, but was still higher than the control (paired-sample t-test). Xylene induced leukocytosis as an increase in absolute neutrophil numbers, solvents reduced erythrocyte counts, haematocrit and haemoglobin. Platelet counts were high throughout the dosing with xylene (Morcos *et al*., 2015). Similar observations were also found byby Ross *et al.* (2002) where a group of mice were inoculated with BaP at certain concentrations (10 mg/kg, 30 mg/kg, 90 mg/kg) through oral exposure. Examination of whole blood samples revealed a dose related decrease for the red blood cell count (RBC), haemoglobin (HB) and haematocrit in 10, 30, and 90mg/kg. The mean cell volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC) were minimal but significantly increased. It also indicates a dose related toxicity for red blood cells in the bone marrow. The decrease in white blood cell count (WBC) in 190 mg/kg treatment was due to a decrease in the number of lymphocytes and eosinophils while absolute number of neutrophils and monocytes remained at control levels. The targets for benzo[*a*]pyrene toxicity are the proliferating haematopoietic cells of the bone marrow (Larsen, 2013).

The result in Tables 4.10 – 4.13 revealed varying toxic effects in the biochemical indices of theWistar albino mice at different doses of the aromatic hydrocarbons analyzed after 35 - days' study period The control values were within the reference range but lower than the treatment groups. There were extremely significant differences detected using two - way ANOVA and Dunnett's multiple comparison test among the dose effect of the treatment group of aromatic hydrocarbons and their controls ($P< 0.05$) but not significant among the biochemical indices of xylene and pyrene treatment groups ($P >0.05$) (Appendix VIo). The results are similar to the research done by Morcos *et al.* (2015), who reported that toxic effect of xylene on the liver was observed in all doses, showing maximum harmful effect at the highest concentration of 0.75 mg/kg. These changes included deviations in liver function markers, with an increase in ALT, AST, total bilirubin, and AFP, accompanied by a decrease in total protein and albumin levels. Renal function markers (urea and creatinine) were elevated after 0.5 mg/kg of xylene injection.The most common liver function tests include serum aminotransferases, total bilirubin, total proteins and albumin, and are commonly employed in clinical practice related to screen for liver disease. Hepatocellular damage results in release of these enzymes into blood circulation. Increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, infarction and muscular damages. ALT is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are indicators of cellular infiltration and functional disturbance of liver cell membranes. On the other hand, bilirubin and albumin values are associated with the function of hepatic cells (Rezaei-Moghadam *et al*., 2012).

The results in in Plates 4.1a – e - 4.4a – e revealed mild and minor morphological changes having congested and oedematous lung with alveolar space containing red cell, gastric mucosa of the stomach having acute and chronic inflammatory cells due to gastritis,

hepatocyte of the liver undergoing necrosis having a sharp contrast with macrovesicular steatosis – fatty liver disease and renal tubular epithelial cells off the kidney undergoing necrosis and the presence luminal calcific casts in comparison to the control with normal morphological architectures at the different doses of the aromatic hydrocarbons.There was no no – observable – effect-level (NOEL) in all the control organs. Xylene has its lowest – observable – effect – level (LOEL) at 1000 mg/kg and no – observable – effect-level (NOEL) at its lowest concentration (400 mg/kg); anthracene has its lowest – observable – effect–level (LOEL) at its lowest concentration (100 mg/kg); pyrene has its lowest – observable – effect – level (LOEL) at its lowest concentration (50 mg/kg) and benzo (a) pyrene has its lowest – observable – effect–level (LOEL) at its lowest concentration (20 mg/kg). The results are similar to the results reported by Issa and El-Sheriff (2015) who opined that anthracene induced disrupted lung architecture with collapsed alveoli, inflammatory cellular infiltration, congested thickened pulmonary vessels and extravasated red blood cells. WHO (2003)reported that male and female CD-1 mice (20 per sex per group) given pyrene by gavage at doses of 0, 75, 125, or 250 mg/kg of body weight per day in corn oil for 13 weeks exhibited kidney effects (renal tubular pathology, decreased kidney weights). The low dose (75 mg/kg of body weight per day) was considered the no – observable – adverse – effect – level (NOAEL) for nephropathy and decreased kidney weights.

Health Canada (2016) reported that in a study in which male and female F344 rats were given BaP in their diet at 0, 5, 50 or 100 mg/kg bw per day for 90 days, an increase in tubular casts in the kidneys of males was observed at the middle and high doses, but no effects were observed in females at any dose. The liver is the main target of toxicity of several compounds. This is because 75 % of blood coming into the liver arrives directly from the gastrointestinal organs and then from the spleen through portal veins, which bring drugs and

xenobiotics in concentrated form. The pathophysiological mechanisms of hepatotoxicity include both hepatocellular and extracellular mechanisms (Issa and El-Sheriff, 2015). Animal studies also indicate that xylene isomers generally induce a wide variety of hepatic enzymes, as well as increased hepatic cytochrome P_{450} content, liver enlargement and other minor hepatic histopathological changes. These types of changes have not been reported to manifest as adverse hepatic effects but rather reflect the liver's increased metabolic activity that occurs in response to xylene exposure. Thus, such effects are considered adaptive changes rather than adverse health effects (Alberta Environment, 2004). Wang and Xue (2015) reported that benzo – (*g, h, i*)-perylene, benzo-(*a*)-pyrene (10 mg/kg), benzo-(*a*)-pyrene (20 mg/kg), and anthracene (50 mg/kg) induce cancer and precancerous lesions in the liver, stomach, and kidney after 90 days of exposure. Hence, the observed effects in this study were mild, minor and minimal pathologicallyadaptive changes which when prolonged (more months and years) could have potential to develop into obvious major, adverse or carcinogenic pathological changes in the sequence order: benzo (a) pyrene (5 rings) > pyrene (4 rings) > anthracene (3 rings) > xylene (1 ring) which agree with the opinion of Moorthy *et al.* (2015) that the carcinogenicity of PAHs is associated with the complexity of the molecule (i.e. increasing number of benzenoid rings).

It has been established by many studies that bioremediation i.e. the exploitation of microorganisms for detoxifications of heavy metal ions, aromatic hydrocarbons, petroleum products, pesticides and other toxic organic molecules is the method of choice owing to fewer secondary hazards and generally low cost. Studies showed that hydrocarbon degrading bacteria are ubiquitously distributed in soil and aquatic environments. However, their populations constitute less than 1 % of total microbial communities. Many of the microorganisms proposed for biodegradation and bioremediation have been isolated from

contaminated soils and waters (Athar *et al.*, 2014). In this study, the result in Table 4.14. showed that surface water harbours more heterotrophic bacteria than the sediment samples which could possibly be due to the more nutrient and oxygen levels in surface water while the result in Table 4.15 revealed that sediment surface harbours more hydrocarbon utilizing bacteria than surface water possibly due to the high accumulative nature of sediment to aromatic compounds than water. There were statistically significant counts detected using ordinary one-way ANOVA and Tukey's multiple comparison test among samples of the three sampled locations ($P < 0.05$) (Appendix VIp). Aromatic compounds tend to have low solubility to water hence making them adsorb more to sediment than surface water. Comparatively, there was significant abundance of THBmore than THUBand more xylene degraders than anthracene and pyrene degradersin both samples, respectively. The result corroborates the findings of Chikere *et al.* (2009), who reported that the waterways are unceasinglyopen to petroleum hydrocarbons owing to navigational actions and this possibly could have augmented the sediment with THUB. However, the paucity of the HUB counts may be attributed to the lack of nutrients at that depth especially nitrogen and phosphorus which get reduced with input of hydrocarbons. Another factor that reduces available metabolic nutrients in marine ecosystem according to Xu *et al.* (2004), is substantial percolation initited by tidal flood and wave acton. The existence of the hydrocarbon utilizers in the midst of the heterotrophic population in the samples is a sign of earlier contamination owing to hydrocarbon pollution (Al-Thani *et al.*, 2009; Esedafe *et al.*, 2015).

The result in Appendices IIIa – IIIc showed that the 48 marine bacterial isolates obtained from Abonema,Nembe and Onne sampling locationshad varying growth patterns with +++ as heavy growth, ++ as moderate growth and + as weak growth in the presence of the substrates namely xylene, anthracene and pyrene. The resultsin Table 4.16 – 4.18 indicated thata total of

nine (9) isolates ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 out of the 48 isolates (9/48) representing 18.75 % of the isolates were screened and selected as best and strongest degraders of xylene, anthracene and pyrene hydrocarbons which they utilize as source of carbon and energy and is revealed by absorbance values of each isolates. This study agrees with the explanation of Mao *et al*. (2012) that the most important aspect of microbial degradation of PAH is enrichment and isolation of indigenous PAH degraders because the indigenous PAH degrading bacteria are already adapted to utilizing PAH. Pathak and Bhatnagar (2011) argued that enrichment culturing is important for the success of hydrocarbon bioremediation because the process leads to selection of microorganisms accustomed to hydrocarbon degradation. Esedafe *et al*. (2015) reported that an occurence of 3/41 representing 7.32 % isolates from refinery effluent were capable of utilizing phenanthrene and anthracene as sole carbon and energy sources. It also indicates that only these isolates had the physiological capabilities to metabolize the various aromatic hydrocarbons.

The results in Appendix IIIdand Table 4.19 indicated that many colonies with different morphological features were isolated on the enrichment medium agar plates after incubation for 14 days. The results of this research agree with the research carried out by Al-Thani *et al.* (2009), who reported that a diverse microbial population can be isolated from hydrocarbon contaminated samples. In agreement with research carried out by Arulazhagan *et al*. (2011) and Akinbankole *et al.* (2015) who reported that bacteria isolated from pyrene and anthracene enriched medium are known to utilize pyrene and anthracene as their sole carbon source for growth and energy. The result in Table 4.20 also indicated that most marine bacterial isolates were Gram negativerod - shaped arranged in singles or pairs, with variable reactions to different to different biochemical tests. These findings agreed with the reports of Mrozik *et*

al. (2003), Okerentugba and Ezeronye (2003), Chikere *et al*. (2009), John *et al.* (2012), Irshaid and Jacob (2015), Wanjohi *et al*. (2015), Isiodu *et al.* (2016) and Fagbemi and Sanusi (2017), that two-third of most petroleum hydrocarbon degraders are Gram negatives with one-third being Gram positive but contradict the findings of Akinbankole *et al*. (2015), Kafilzadeh *et al*. (2012) and kafilzadeh and Pour (2012), who reported that more of Gram positive bacteria were isolated than Gram negative bacteria. A lot of rod-shaped bacteria have also been implicated in hydrocarbon degradation studies (Okoh(2003); Perfumo *et al*. (2007); Chikere *et al*.(2009); Alfreda and Ekene (2012); John *et al.*, (2012); Akinbankole *et al*.(2015); Irshaid and Jacob (2015); Wanjohi *et al*.(2015); Isiodu *et al.*(2016) and Fagbemi and Sanusi (2017)) and similar results were obtained in this study.

Molecular characterization of bacteria helps in the quantification and detection of their phylogenetic diversity. Molecular identification of bacteria is highly sensitive and specific as compared to a biochemical approach of identification. Molecular characterization of the 16S rRNA gene using polymerase chain reaction (PCR) is a well-known method of identifying a species and genera of bacteria (Srujana and Khan, 2012). The result in Plate 4.5 confirmed that the PCR products had the molecular weight of 1 kbp as evidenced by UV fluorescence under agarose gel electrophoresis. Akinbankole *et al.* (2015) obtained in their research 1,500 bp PCR product from anthracene and pyrene isolates isolated from oil contaminated water and soil in Malaysia. Yuliani *et al.* (2012), obtained in their research 1,489 bp PCR product from phenanthrene and pyrene isolates from marine area of Indonesia. Isiodu *et al.* (2016) reported that all the seven (7) polyaromatic hydrocarbon - utilizing bacterial isolates from Bodo Creek brackish water in Nigeria showed amplification with an amplicon size of 500 bp. The results in Appendices IVa –IVi indicated that there werevariable nucleotide sequences of the nine (9) marine bacterial degraders.The result in Table 4.21 also indicated that the nine (9) marine bacterial degradershad 90 – 100 % homologus sequences with their counterparts

in the GenBank after blasting at NCBI website. Akinbankole *et al*. (2015) found out that *B. cereus* was the most blasted organism with sequence homology (99 %). The phylogenetic tree depicts the evolutionary correctional among hydrocarbon metabolizing bacteria isolated from the three (3) sampling sites as described in this study. The result in Figure 4.12 revealed that the nine (9) marine bacterial degradershad similar lineage as they arise from the same analogous node and hence they are evolutionary connected to their relatives in the GenBank. The nucleotide sequences of these genes have been submitted to NCBI/ GenBank database since first of December,two thousand and sixteen (01/12/16). They belong to the genera of*Providencia*, *Alcaligenes*, *Brevundimonas*, *Myroides*, *Serratia*, *Bacillus*; and families of Enterobacteriaceae, Alcaliginaceae, Caulobacteraceae, Flavobacteriaceae, and Bacillaceae; and phyla ofProteobacteria, Bacteroidetes and Firmicutes which members have been implicated in petroleum and aromatic hydrocarbon biodegradation by several authors (Chikere *et al.*, 2009; John *et al.*, 2012; Yuliani *et al.*, 2012; Athar *et al.*, 2014; Ichor *et al.*, 2014; Swaarthy *et al.*, 2014a; Amer *et al.*, 2015; Akinbankole *et al*., 2015; Irshaid and Jacob, 2015; Louvado *et al.*, 2015; Patowary *et al.*, 2015b; Isiodu *et al*., 2016; Fagbemi and Sanusi, 2017).

The results in Figures 4.13 – 4.15 indicated that the utilization and degradation of these compounds after 5 days of incubation resulted in increase in optical density (cell mass) of the organisms; however, increase in concentration of these compounds led to decrease in optical density (cell mass) of the organisms with very strongly significant negative correlation ($P < 0.05$; $r = -0.783 – 0.980$) (Appendix VIq). It is apparent from the results that strains isolated on xylene (*Alcaligenes faecalis*XYL2 and *Providencia* sp. XYL8) and anthracene (*Providencia vermicola*ANT1) were able to grow better on the three tested aromatic hydrocarbons than all the strains (*Brevundimonas diminuta* PYR3, *Alcaligenes faecalis* PYR5

and *Bacillus cereus* PYR9) that were isolated on pyrene.Generally, all the nine strains (9) degraded all the three aromatic hydrocarbons and grew well indicating multiple biodegradation potentials but with different efficiencies, hence termed multiple degraders. Also, these strains especially *Alcaligenes faecalis*XYL2and *Providencia* sp. XYL8 degraded xylene and anthracene equally but less than pyrene hydrocarbons in the order of degradation: xylene > anthracene > pyrene. Comparatively, *Providencia* sp. XYL8, *Providencia vermicola*ANT1 and *Alcaligenes faecalis*XYL2 had better degradation efficiencies than the restof the other six (6) strains (*Brevundimonas diminuta* PYR3, *Alcaligenes faecalis* ANT4, *Alcaligenes faecalis* PYR5, *Myroides odoratus* ANT6, *Serratia marcescens* XYL7 and *Bacillus cereus* PYR9).There were extremely significant differences detected using ordinary one - way ANOVA and Dunnett's multiple comparison test among the treatment groups of the cell growth suspensions and the concentration of hydrocarbons ($P< 0.05$) (Appendix VIr). The result is consistent with the research carried out by Poornachander *et al*. (2016), who reported that growth of *Bacillus cereus* CPOU13 decreased with increasing PAH concentrations (phenanthrene, anthracene and pyrene) from 10 ppm to 250 ppm in MSM. Also, similar was the work carried out by John *et al.* (2012), in which they found out that the growths of all the test isolates (*Alcaligenes faecalis* AFS-5, *P. putida* AFS-3 and *M. varians* AFS-2) were PAH-dependent and provide strong evidence for selective PAH degradation by bacteria. The acclimation of microbial community to one substrate, may lead to the simultaneous acclimation to some but not all structurally related molecules. Akinbankole *et al.* (2015), isolated and identified *B. thuringiensis, B.megaterium* and *B.cereus* in both pyrene and anthracene enriched medium and the three bacteria have the metabolic adaptability of utilizing low and high molecular weight PAH. Their abilities to utilize both low and high molecular weight PAHs is an indication of the possession of ring fission enzymes (Amund *et al.*, 2006).

The result in Table 4.22 indicated that the abilities of the isolates to degrade different hydrocarbons were found to vary and these differences could be attributed to the membrane toxicity and non-possession of the necessary enzymes (Igwo-Ezikpo *et al.*, 2006). The result agreed with report of Fagbemi and Kehinde, (2017) that the abilities of the bacterial hydrocarbon degraders to degrade hydrocarbons varied and *C. koseri, S. ficaria* and *B. coagulans* had moderate/strong growth on crude oil which is similar in this study but differ in species used (*Serratia marcescens* XYL7 and *Bacillus cereus* PYR9). John and Okpokwasili (2012) report that nitrifying bacteria are excellent degraders of crude oil and has ability to utilize crude oil and its products. The result in Table 4.23 indicated that all the isolates had growth and resisted all metals except *Brevundimonas diminuta* PYR3, *Alcaligenes faecalis* ANT4 and *Myroides odoratus* ANT6 that were sensitive (–) to AsO_3 and CdO compounds. The result agreed with the report of Jaysanker *et al.* (2007) that single bacterial strains can be resistant to many metals and that the multi-metal resistant bacteria highly resistant to mercury possess the genetic components for dealing with many toxic metal ions. These isolates are of interest for molecular characterization of mechanisms for resistance to multiple metals and hold promise for bioremediation of toxic heavy metals, including in environments that are contaminated by several metals. Also, Athar *et al*. (2014) published that the aromatic hydrocarbon degrading bacterial isolates were capable of degrading a variety of different hydrocarbons with its ability to grow in different metals stress environment. It was observed that all the bacteria sensitive to the metal compounds ($PbNO_3$, $ZnCl_2$, $COCl_2$, $CdCl_2$, $K_2Cr_2O_7$, Hg and $NiCl_2$) have shown sensitivity even at lowest usedconcentration (50 mg/ml), whereas the resistant bacteria showed resistance to the highest concentration (150 mg/ml).

The results in Tables 4.24 – 4.26 indicated that the degree of weight losses were observed to increase with increase in incubation period but varied with different microbial species tested after 24 - days' biodegradation study with evidence of increasingly low optical density (OD_{600} nm). However, the level of xylene, anthracene and pyrene degradations also included 5.20 ± 0.03 %, 4.60 ± 0.01 %, 11.10 ± 0.06 % degradation by abiotic factor as observed in controls with no bacterial inocula. After deduction of the aromatic hydrocarbons degradation by abiotic factor, 94.30 ± 0.02 % and 49.90 ± 0.06 % of xylene and pyrene degradation was in fact contributed by *Serratia marcescens* XYL7 and 92.80 ± 0.02 % by *Alcaligenes faecalis* PYR5 during this period. The differences in xylene, anthracene and pyrene degradation abilities of these bacterial strains were clearly reflected even after 4- days of incubation which was further magnified during incubation periods (4 – 24 days) with very strongly significant positive correlation ($p < 0.05$) (Appendix VIs). There were extremely significant differences detected using ordinary two - way ANOVA and Dunnett's multiple comparison test among the treatment group of incubation daysand the aromatic hydrocarbon degradations by the degraders and their controls ($P < 0.05$) (Appendix VIt). Thus, xylene, anthracene and pyrene were degraded by all nine bacterial strains, but they differed widely in their inherent abilities.The differences might be due to high molecular weight PAHs that are more recalcitrant and hard to microbial attack and the sequence of degradation is xylene > anthracene > pyrene. Biodegradation of PAHs is depended on their chemical structure and corresponding physiochemical properties and low molecular weight PAHs degrade rapidly than high molecular weight PAHs (Guo *et al.,* 2005). Similar results are obtained in the present study. Singh *et al.* (2013) reported that *Rhodococcus pyridinvorans* NJ2 was the highest degrader (60 %) of pyrene, followed by *Pseudomonas* sp. BP10 (44 %) and the least was *Ochrobactrum intermedium* P2 (42 %) in MSM with pyrene (50 µg/ml) in 8 - days. Swaathy *et al.* (2014a) reported that the marine isolate, *Bacillus licheniformis* MTCC 5514

degraded > 95 % of 300 ppm anthracene in an aqueous medium within 22 – days and the degradation percentage reduced significantlywhen the concentration of anthracene increased to above 500 ppm. Akinbankole *et al.* (2015) reported that pyrene and anthracene utilizing bacteria (*Salmonella enterica* and *Bacillus toyonesis*) were isolated from water and used engine oil contaminated soil degraded 99 % of the PAH within seven days. Arulazhagan *et al*. (2011)in their report in which bacteria they isolated from contaminated sites also degraded over 99 % of flourene and anthracene in seven days. Poornachander*et al*. (2016) reported that *Bacillus cereus* CPOU13 degraded phenanthrene to 73.46 % and its initial concentration declined from 216.32 µg to 56.57 µg; anthracene to 85.76 % and its initial concentration reduced from 209.20 µg to 32.63 µg and pyrene to 47.88 % and its initial concentration reduced from 230.14 µg to 119.95 µg. Qi *et al*. (2017) reported that *Gordonia* sp. nov. Q8 could remove 73.8 % and 53.4 % of anthracene and pyrene in 7 - days. Lily *et al*. (2013) reported that *Brachybacterium paraconglomeratum* strain BMIT637C was the efficient degrader of anthracene being capable of degrading 70.32 % of anthracene within 10 – days showing 2 x 10^{45}-fold increase in the CFU number that indicated anthracene utilization as a sole source of carbon and energy. Akpe *et al.* (2013) reported that the lower the optical density value, the higher the percentage degraded. Pandey *et al.* (2012) reported that PAH (phenanthrene, flourene, anthracene, pyrene) dissipation level ranged between 38.7 and 99.7 % with highest depletion recorded in phenanthrene within seven days. The degradation rate of 3-ring PAH was higher as compared to 4-ring PAH (pyrene) by *Serratia marcescens* L-11.The findings of these authors corroborated with the results of this study.

The results shown in Plates 4.6a – b - 4.8a – band Appendix Vdemonstrated that the 100 mg/l of xylene, anthracene and pyrene from the assay medium were degraded and reduced into lesser, smaller and lighter fragments/sizes with clear confirmations of biodegradation losses

of some aromatic hydrocarbons components. These losses, reductions or disappearances in spot sizes could be attributed to the usage of aromatic hydrocarbons by marine isolates during the 24 - days' incubation period under ideal cultural settings of temperature and pH as sole carbon and energy sources. Moreover, the results were in conformity with the results of the degradation kinetics of the hydrocarbons by the marine bacterial isolates at day 24 with increasingly low ODmonitored at 240 nm and is in agreement with published work of Bennet *et al*. (2012),who reported that the thin layer chromatography of test anthracene showed small developed spots with low intensities when compared with control visualized at 235 nm under UV illuminator. Previous study reported that the thin layer chromatographic study of the purified metabolites after the pyrene degradation course showed the existence of a single spot with Rf value of 0.65, which is similar to the reference Rf value of protocatechuic acid and conformed with the pyrene Rf value in this study (Teh and Hadibarata, 2014). Also, in agreement with Akinbankole *et al.* (2015) the disapperance of pyrene and anthracene in the medium indicates that the pyrene and anthracene in the medium have been metabolized by these bacteria.

The results in Tables 4.27 – 4.29 and Figures 4.16 – 4.21 demonstrated that metabolites I (m – toluic acid, paranaphthalene and 9, 10 - dihydroanthracene) eluted at 6.30, 9.82 and 11.61 minutes had the highest base molecular ion peak of 136 (100), 236 (100) and 180 (100) m/zs, and other major fragment ions at m/z values of 91 (97) and 119 (57); 221 (30) and 193 (28) and 179 (94) and 178 (51.5) while metabolites VII, VI and IV (acetaldehyde, benzenecarboxylic acid and hydroxylmethylbenzene) had the lowest base molecular ion at 29 (100), 105 (100) and 79 (100) m/zs with 6.04, 5.54 and 4.58 minutes retention time and the other major fragment ions at m/z values of 44 (82) and 43 (48); 122 (84) and 77 (67.5); and 108 (89) and 107 (71), respectively during xylene, anthracene and pyrene degradations.

These mass fragments were in agreement with published spectra of Kim *et al.* (2016). Kim *et al*. (2015) reported that the degradation of *m*-xylene to 3 - methylbenzoate and 3 - methylcatechol with further cleavage to propionaldehyde and pyruvate. Morasch *et al*. (2004) reported that 3 - methylphenylitaconic acid, *m*-toluic acid and methylbenzylsuccinate were extracted from supernatants of *m* – xylene grown cultures. Otenio *et al.* (2005) reported the degradation of *m* -xylene to 3 - methylbenzenealcohol and Lakshmi and Velan (2013)reported four metabolites having base ion at m/z 73 and the other specific ion at m/z 147 from anthracene extracts. Ahmed *et al*. (2012) observed the formation of anthrone by alkaliphilic bacteria at C9 and C10 positions and further leads to the formation of quinone product of PAH and two quinone products were observed in this study. Swaathy *et al*. (2014a) found out that their mass spectral analyses and the librarydetails revealed that (i) naphthalene (m/z - 128), (ii) naphthalene- 2-methyl (m/z - 142), (iii) benzaldehyde-4-propyl (m/z - 148), (iv) 1, 2, benzene di-carboxylic acid (m/z - 167) and (v) benzene acetic acid (m/z - 137) were the major degraded products detected. The pathways of the aromatic hydrocarbon degradation were proposed based on the metabolites obtained from mass spectra analyses. The aromatic hydrocarbon metabolism pathways vary depending on the species used in this degradation study. Furthermore, it seems likely that the degradation of individual aromatic hydrocarbon compounds by the selected bacteria proceeds via independent pathways (Hesham *et al.*, 2014). From the metabolic pathway of these bacteria, it could be concluded that these strains utilized 100 mg /l of xylene (one ring), anthracene (three rings), and pyrene (four rings) via two main pathways and catechol is the major constant product that appeared during the degradation period (24 – days). Thus, xylene, anthracene and pyrene degradation by strains *Serratia marcescens* XYL7 and *Alcaligenes faecalis* PYR5 produced several metabolites which are similar to the ones produced by other xylene, anthracene and pyrene degraders such as catechol by *Leclercia adecarboxylata* PS4040 (Sarma *et al.*, 2010),

phthalic acid by *Sphingomonas koreensis* strain ASU-06; *Alcaligenes faecalis* MVMB1, *P. vulgaris* 4Bi and *P. fluorescens* 29 L (Hesham *et al.,* 2014; Lakshmi and Velan, 2013; Ceyhan, 2012; Husain, 2008; Peng *et al.*, 2008); toluic acid by *Desulfotomaculum* (Morasch *et al*., 2004) and benzylalcohol by *P. putida* CCMI 852 and *Pseudomonas putida* mt-2 (Otenio *et al.*, 005; Kim *et al*., 2015) and 9, 10 – dihydroanthracene (9,10 – anthraquinone) by marine *Bacillus licheniformis* MTCC 5514 (Swaathy *et al*., 2014a; Peng *et al.*, 2008).

Inorder to broaden the description of the aromatic hydrocarbon - degrading bacteria isolated from the Rivers State marine environment, PCR assays were performed to explore for functional genes coding for the catechol 2, 3 - dioxygenase enzyme (*C23O*) and genes related to surfactant production namely rhamnolipid enzyme (*rhlB*) and surfactin/lichenysin enzyme (*SrfA3/LicA3*) and the results are presented in Table 4.30and Plate 4.9. From the results, catabolic gene (*C23O*) was detected in only four (4) out of the nine marine aromatic degrading bacteria with names stated above while surfactant genes (*rhlB, SrfA3/LicA3*) were detected only in three (3) out of the nine marine aromatic degrading bacteria all of which had 881 base pairs sizes of PCR products of the catabolic and surfactant genes. Even though, biosurfactants help in solubilizing or mediating the interface between the marine bacterial degraders and the aromatic compounds, the catabolic responses detected in our study were effected by the dioxygenase genes as revealed from the amplified product of 881 bps.The detection of *C23O* gene in some of the Gram negative bacteria and not in the Gram positive bacteria showed that these strains (*Providencia vermicola*ANT1, *Alcaligenes faecalis*XYL2, *Serratia marcescens* XYL7and *Providencia* sp. XYL8) synthesize dioxygenases for either complete or partial breakdown of aromatic hydrocarbons (xylene, anthracene and pyrene) and a possible reason could be that the detected genes are highly preserved among diverse Gram-negative bacteria and is in conformity with the research work of Hesham *et al*. (2014), who

confirmed the existence of both monooxygenase and dioxygenase in *S. koreensis* strain ASU - 06. *C12O* and *C23O* dioxygenases have been known to contribute a significant part in the catabolism of aromatic rings by the bacteria as they are responsible for splitting of aromatic C – C bond at ortho or meta positions. The implication of the gene *C23O*as an essential gene in the catabolism of low and higher molecular weights PAHs has been reported by Swaathy *et al.* (2014a). Moreso, the detection of rhamnolipid (*rhlB*) and surfactin/lichenysin (*SrfA3/LicA3*) genes in these strains upheld the continuous argument among the scientists about the occurence of *rhlB* and *SrfA3/LicA3*genes in bacteria other than *Pseudomonas* and *Bacillus* species and several new strains of bacteria having exceptional ability to produce rhamnolipids have in recent times been described. There is dearth of reports documented on rhamnolipid production among pathogens including *Burkholderia mallei* and *B. pseudomallei* and the non-pathogenic especially *B. thailandensis.* This observation contradicts the findings of Swaathy *et al.* (2014a and 2014b), who found out that the expression of *srf* and *licA3* genes were only realized in all of the five *Bacillus* species and marine *Bacillus licheniformis* MTCC 5514. On the other hand, those bacterial isolates that could grow on the aromatic hydrocarbons but had unsuccessful amplification, could be as result of incompatibilities among the tested primers and gene sequences (Ding *et al.*, 2010) and may contain other catabolic and surfactant genes aside the tested genes.

Catabolic pathways which encoded different aromatic hydrocarbon degradation routes, are frequently located on plasmids, although degradative genes can be located on either chromosome or plasmid (Coral and Karagoz, 2005). In our study, plasmid profile and curing experiment was carried out to invetsigatefor the presence of degradadive bacterial genes on plasmid or chromosome and the result in in Table 4.31 revealed that all the nine marine bacterial strains that were successfully cured by sub – culturing in Nutrient broth lost the

ability to degrade aromatic hydrocarbons after plasmid curing when grown on MSM with xylene, anthracene and pyrene as carbon sources. It is assumed that this may be assumed that this may be because of the removal/inactivation of gene (s) responsible for aromatic hydrocarbon degradation from the bacterial strains. Curing experiment demonstrated that strains that lost their plasmids also lost their hydrocarbon biodegradation ability, so the capacity to degrade hydrocarbons is plasmid - related in all the nine (9) strains and is similar to the findings of Fagbemi and Sanusi (2017). The curing experiment was verified by the screening by screening for the presence of degradative bacterial genes on plasmid or chromosome using antimicrobial susceptibility testing of the isolates and the result in Table 4.32 suggests that the marine bacterial strains may possess the resistance genes for these two antibiotic (ampicillin and amoxicillin) and hence, these two antibiotics were used as markers for the screening of plasmid cured bacterial colonies. Similar observation was obtained by Kumar *et al*. (2010) who tested several antibiotics for their resistances against *Pseudomonas* sp. strain E and observed that strain E was resistant against cefadroxil and ampicillin antibiotics. John and Okpokwasili(2012) reported that *Nitrosomonas* and *Nitrobacter* species were resistant against cefadroxil and ampicillin antibiotics. In order to check for the purity of the extracted plasmids as well as determine the number and size of the plasmids harbouring the catabolic genes involved in aromatic hydrocarbon degradation, electrophoretic profiles of plasmids isolated from the cured strains were compared with those of the non – cured ones (Plate 4.10). The result in Plate 4.10 showed that the plasmids were partially cured (multiple plasmids) hence the incomplete disappearance of all the bands after plasmid curing in the seven isolatesexcept *Brevundimonas diminuta* PYR3 and *Alcaligenes faecalis*PYR5with complete curing (single plasmids).Although there was partially curing, it further revealed that catabolic genes responsible for xylene, anthracene and pyrene degradations were located on the plasmids. It can be deduced that their presence in polluted substrate encourages the

development of adaptivefeatures such as plasmid which support hydrocarbon cometabolism (John and Okpokwasili, 2012). The presence of multiple plasmids in hydrocarbon degrading bacteria were already reported in *A. chroococcum* (Thavasi *et al.*, 2006), *Klebsiella pneumoniae* and *Serratia marscencens* (Akpe *et al.*, 2013), *Pseudomonas* species (Jyothi and Umamaheswara, 2009) and *Enterobacter cloacae* (Esumeh *et al.*, 2009). Rasool *et al.* (2003) reported that most of the cured isolates had lost the potential *in toto* to degrade xylene and octane while majority of the cured isolates were only partially cured to degrade naphthalene. Infact, a complete or partial curing is the function of the copy number of plasmids plus the scattered distribution of these copies (the ones residing away from the periphery are not affected by the curing agents). Similar observation was reported by Fagbemi and Sanusi (2017) who reported that the genetic factor responsible for crude oil degradation in *Bacillus coagulans* and *Citrobacter koresi* were chromosomal - mediated while that of *Serratia ficaria* was plasmid - mediated.

5.2 Conclusion

The present study revealed that the three sampling sites namely Abonema Wharf, Nembe waterside and Onne Light Terminal of the Rivers State marine environment contain higher quantities of aromatic hydrocarbons, heavy metals and other physico-chemical parameters in the sediment samples than water samples.

These toxicity results (EC_{50}/ErC_{50}/LC_{50}/LOEL) are in line with other toxicity values for this type of pollutants, and are considered being scientifically relevant in ecotoxicological risk assessment and the environmental public health effects of these pollutants should be given attention.

The results also suggest that the studied areas harbour a lot of excellent and efficient bacterialdegraders especially *Serratia marcescens* XYL7 which couldplay an important role in biodegradation of simple, low and high molecular weight aromatic hydrocarbons and hence be exploited in the bioremediation campaigns for aromatic hydrocarbon - contaminated ecosystems in the Niger Delta.

5.2.1 Contribution to Knowledge

This study contributed to filling the gap of knowledge on the toxicological profiles of aromatic hydrocarbons in Rivers State marine environment revealingthe potential environmental and animal health effects/implications of these pollutants/contaminantsand the urgent need to improve health policies, launch aromatic hydrocarbon - related health and toxicity awareness campaigns, inorder to get rid of their dangerous outcomes.

It also contributed to filling the gap of knowledge on the microbial diversity of Rivers State marine environment revealing the potential of these ecosystems as reservoirs of excellent aromatic hydrocarbons bacterial degraders and their possible exploitation for future *in situ*and *ex situ*intervention to combat pollution in the contaminated ecosystems in the Niger Delta.

Furthermore, the study widens our knowledge on the genetics of bacterial degraders from the Rivers State marine environment as the plasmids of these strainsespecially *Serratia marcescens* XYL7 could be used in recombinant DNA technology to develop bacterial cells with potential to degrade aromatic hydrocarbons.

5.3 Recommendations

Ecotoxicological assays involve batteries of assays comprising acute and chronic toxicity bioassays representing different trophic levels. Further studies on chronic toxicity assays should be carried out to augment the batteries of acute toxicity assays conducted in this study inorder to obtain a comprehensive data for ecotoxicological risk assessment of River State marine sediment and water samples.

The biases associated with culture - dependent microbial enumeration techniques limited the full description of the bacterial diversity in this study. Further studies should integrate meta - genomic technologies as well as microbial community analysis in order to increase our understanding of the vast microbial diversity under the waters in the oil rich Rivers State, Nigeria.

Also, PCR assays should be performed to explore for more functional genes coding for other catabolic and surfactant enzymes aside the tested primers used in this study in order to furtherwiden the characterization of the aromatic hydrocarbon - degrading bacteria isolated from the Rivers State marine environment.

REFERENCES

Abdel-Shafy, H. I. and Mansour, M.S.M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25: 107 – 123.

Abu, G.O. and Chikere, B.O. (2006). Cell surface properties of hydrocarbon utilizing bacterial isolates from the Port Harcourt marine environment. *Nigerian Journal of Microbiology*,**20** (1): 809 – 816.

Adams, G. O., Fufeyin, P. T., Okoro, S.E. and Ehinomen, I. (2015). Bioremediation, biostimulation and bioaugmention: A Review. *International Journal of Environment, Bioremediation and Biodegradation*, **3** (1): 28 – 39.

Adenike, A. (2014). Microbiological, physicochemical and genotoxicological assessment of tobacco wastewater. *Advances in Applied Science Research*, **5** (3):423 – 438.

Ahmad, D. M., Juahir, H., Yunus, K., Amran, M.A., Hasnam, C.N.C., Azaman, F., Abidin, I. Z., Azmee, S.H. and Sulaiman, N.H. (2015). Oil spill related heavy metal: a review. *Malaysian Journal of Analytical Sciences*, **19** (6): 1348 – 1360.

Ahmed, A., Choi, C.H., Choi, M.C. and Kim, S. (2012). Mechanisms behind the generation of protonated ions for polyaromatic hydrocarbons by atmospheric pressure photoionization. *Analytical Chemistry*, **84**: 1146 – 1151.

Akinbankole, A.S., Tunung, R. and Tennant, A.M. (2015). Biochemical and molecular characterization of pyrene and anthracene metabolizing bacteria isolated from oil contaminated water and soil in Malaysia. *Journal of Applied and Environmental Microbiology*, **3** (1): 25 – 30.

Akpe, A.R., Ekundayo, A.O. and Esumeh, F.I. (2013). Degradation of crude oil by bacteria: A role for plasmid-borne genes. *Global Journal of Science Frontier Research,* **13** (6): 20 – 26.

Alberta Environment, (2004). Assessment report on xylenes for developing ambient air quality objectives. Cantox Environment Incorporation and RWDS West Incorporation, Canada. Pp. 3 – 9.

Alfreda, O.N. and Ekene, G.O. (2012). Bioremediation of crude oil polluted soil using bacteria and poultry manure monitored through soybean productivity. *Pollution Journal of Environmental Studies*,**21** (1): 171 – 176.

Aly Salem, D. M. S., Morsy, F. A. E. M., El Nemr, A., El-Sikaily, A. and Khaled, A. (2014). The monitoring and risk assessment of aliphatic and aromatic hydrocarbons in sediments of the Red Sea, Egypt. *Egyptian Journal of Aquatic Research*, **40**: 333 – 348.

Al-Thani, R.F., Abd-el-haleem, D. A. M. and Al-shammri, M. (2009). Isolation and characterization of polyaromatic hydrocarbons-degrading bacteria from different Qatari soils. *Journal of Microbiology*, **3** (11): 761 – 766.

Amer, R.A., Mapelli, F., El-Gendi, H.M., Barbato, M., Goda, D.A., Corsini, A., Cavalca, L., Fusi, M., Borin,S., Daffonchio, D. and Abdel-Fattah, Y.R. (2015). Bacterial diversity and bioremediation potential of the highly contaminated marine sediments at El-Max District (Egypt, Mediterranean Sea). *Biomedical Research International*, **2015**: 981829.

Amund, O.O., Ilori, M.O., Adebusoye, S.A. and Musa, K.J. (2006). Utilization of alicyclic compounds by soil bacteria. *Nature and Science*, **4** (3): 65 – 68.

Ansari, T. M., Marr, I. L. and Tariq, N. (2004). Heavy metals in marine pollution perspective- A mini review. *Journal of Apllied Sciences*, **4** (1): 1 – 20.

AOAC (2012). Official Method of Analysis. 19th edn. Association of Official Analytical Chemists, Washington DC, USA. Pp.121 – 130.

APHA (2012). Standard Methods for Examination of Water and Wastewater. 22nd edn. American Public Health Association, Washington, DC, USA. P. 1360.

Arulazhagan, P., Yeom, I.T. and Vasudevan, N. (2011). Biodegradation of polycyclic aromatic hydrocarbon by a halotolerant bacterial consortium isolated from marine environment. *International Journal of Environmental Science and Technology*, **7** (4): 639 – 652.

Aruoja, V., Sihtmäe, M., Dubourguier, H. C. and Kahru, A. (2011). Toxicity of 58 substituted anilines and phenols to algae *Pseudokirc hneriella subcapitata* and bacteria *Vibrio fischeri*: Comparison with published data and QSARs. *Chemosphere*, **84** (10): 1310 – 1320.

Asker, S. (2011). Ecotoxicological test methodology for environmental screening of the European water framework directive's priority substances adjusted to Swedish

regional conditions. Degree project in biology, Master of Science (2 years), 2011. Biology Education Centre, Uppsala University, and County Administration Board of Blekinge, Karlskrona. Pp. 8 – 113.

ASTM (1992). Standard methods for conducting acute toxicity tests with fishes, macro invertebrates, and amphibians. *In*: Annual book of ASTM standards. Designation E 729 – 88a, American Society for Testing and Materials, Philadelphia, USA. Pp. 403 – 422.

Athar, M.A., Akbar, A., Khan, Y.H., Ali, I., Mehmood, U., Sabri, A.N. and Hasnain, S. (2014). Characterization of hydrocarbon degrading bacteria isolated from hydrocarbon contaminated soil. *Journal of Pure and Applied Microbiology*,**8**(6): 1 – 9.

Ayanda, O.S. (2014). Occurrence, fate and treatment methods of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, dioxins and furans: a mini review. *Research and Reviews: Journal of Material Science,* **2** (4): 14 – 21.

Ayangbenro, A. S. and Babalola, O.O. (2017). A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *International Journal of Environmental Research and Public Health*, **14**: 94.

Ayuba, K.A. (2012). Environmental impact of oil exploration and exploitation in the Niger Delta of Nigeria. *Global Journal of Science Frontier Research Environment and Earth Sciences*,**12** (3): 18 – 28.

Babita, K., Singh, S.N., Deeba, F., Sharma, M., Pandey, V. and Singh, D.P. (2013). Elucidation of pyrene degradation pathway in bacteria. *Advances in Bioresearch,* **4** (2): 151 – 160.

Bahig, A.E., Aly, E.A., Khaled, A.A. and Amel, K.A. (2008). Isolation, characterization and application of bacterial population from agricultural soil at Sohag Province, Egypt. *Malaysian Journal of Microbiol*ogy, **4** (2): 42 – 50.

Bazzi, A.O. (2014). Heavy metals in seawater, sediments and marine organisms in the Gulf of Chabahar, Oman Sea. *Journal of Oceanography and Marine Science*, 5 (3): 20 – 29

Bennet, R.M., Dagamac, N.H.A., Fernandez, E.V.M., Uba, M.O. and Ching, M.W. (2012). In vitro degradation of anthracene by *Mycobacterium* sp. GIPAH-01 isolated from Guimaras Island Phillipines. *Asian Journal of Experimental Biological Science*, **3** (4): 682 – 687.

Bobak, D.M. (2010). Polycyclic aromatic hydrocarbon characterization in Otter Creek, Northwest Ohio. Theses and dissertation submitted as partial fulfilment of the requirements for the Master of Science Degree in Geology, College of Graduate Studies, University of Toledo, USA 2010. P. 795.

Cao, B., Nagarajan, K. and Loh, K.C. (2009). Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. *Applied Microbiology and Biotechnology*, **85**: 207 – 228.

Ceyhan, N. (2012). Biodegradation of pyrene by a newly isolated *Proteus vulgaris*.*Scientific Research and Essays*,**7**(1): 66 – 77.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2, 2nd edn. Cambridge University Press, New York USA. Pp. 38 – 70.

CSP (2016). Chemical risk Assessment: Aquatic toxicity. L. Pro (Ed.) ChemSafetyProPublication. Accessed on 01/03/1997.

Chikere, C. B., Okpokwasili, G. C. and Ichiakor, O. (2009). Characterization of hydrocarbon utilizing bacteria in tropical marine sediments.*African Journal of Biotechnology*, **8** (11): 2541 – 2544.

Coral, G. and Karagoz, S. (2005). Isolation and characterization of phenanthrene degrading bacteria from a petroleum refinery soil. *Annals of Microbiology*, **55**:255 – 259.

Cuong, D. T. (2005). Measurement and behavior of heavy metals in the marine environment of Singapore. A Thesis submitted for the Degree of Master of Engineering Department of Chemical and Biomolecular Engineering, National University of Singapore, Singapore. Pp. 5 – 9.

Dahan, Y.L. (2005). Preparation of vector containing full length β – catenin cDNA and it transfection into WHCO1 cell line. *In*: Y.L. Dahan (Ed.). The actin cytoskeleton and the nuclear translocation of β – catenin in human oesophageal squamous carcinoma cell lines. Wiilliam Cullen Research Library, University of the Witwatersrand South Africa. P.62.

Dasgupta, D., Ghosh, R. and Sengupta, T.K. (2013). Biofilm-mediated enhanced crude oil degradation by newly isolated *Pseudomonas* species. *ISRN Biotechnology,***2013** (250749): 1– 13.

Davoren, M.N., Shúilleabháin, S., O´Halloran, J., Hartl, M. G. J., Sheehan, D., O´Brien, N.M., Van Pelt, F. N. A. M., and Mothersill, C. (2005). A test battery approach for the ecotoxicologicalevaluation of estuarine sediments. *Ecotoxicology*,**14**:741 – 55.

DigitalGlobe, (2018). Geoeye satellite image. DigitalGlobe. www.digitalglobe.com. 02/03/18. Pp. 1 -3.

Ding, G.C., Heuer, H., Zühlke, S., Spliteller, M., Pronk, G. J., Heister, K., Kogel, K.I. and Smalla, K. (2010). Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes by using a novel PCR detection system. *Applied and Environmental Microbiology*, **76**, (14): 4765 – 4771.

Emami, S., Pourbabaei, A.A. and Alikhani, H.A. (2014). Interactive effect of nitrogen fertilizer and hydrocarbon pollution on soil biological indicators. *Springer Environmental Earth Science,* **72**(9):3513 – 3519.

Emoyan, O. O. (2009). Quantification and distribution of polynuclear aromatic hydrocarbons (PAHs) in surface waters in the vicinity of Kokori Oil Field, Nigeria. *Journal of Applied Science and Environmental Management*, **13** (4): 39 – 42.

Environmental Bio-detection Products Incorporation (EBPI), (2016). Biotoxicity/microbiotest products handout and manual. Antario Canada. Pp. 1 – 37.

Esedafe, W. K., Fagade, O. E., Umaru, F. F., and Akinwotu, O. (2015). Bacterial degradation of the polycyclic aromatic hydrocarbon (PAH) -fraction of refinery effluent. *International Journal ofEnvironmental Bioremediation and Biodegradation*, **3** (1): 23 – 27.

Esumeh, F. I., Akpe, A. R. and Eguagie, O. E. (2009). Crude oil degrading capabilities of bacterial isolates from pawpaw (*Carica papaya*) and sweet orange (*Citrus sinensis*). A role for plasmid - mediated gene. Proceedings of the 1st InternationalConference, Workshop and Exhibition on Biotechnologiesfor Improved Production of Oil and Gas inthe Gulf of Guinea, held in Abuja, Nigeria. April 1-3, 2009. BIPOG3-4-34. Pp. 1 – 7.

European Union – EU (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of European Union*,**276**: 33 – 79.

Fagbemi, O. K. and Sanusi, A. I. (2017). Chromosomal and plasmid mediated degradation of crude oil by *Bacillus coagulans, Citrobacter koseri* and *Serratia ficaria* isolated from the soil.*African Journal of Biotechnology*, **16** (21): 1242 – 1253.

Fentiman, A. and Zabbey, N. (2015). Environmental degradation and cultural erosion in Ogoniland: A case study of the oil spills in Bodo. *The Extractive Industries and Society*, **2**: 615 – 624.

FME (2001). National Guidelines and Standard for Water Quality in Nigeria.Federal Ministry of Environment, Nigeria. P. 114.

GESAMP (1997). Towards safe and effective use of chemicals in coastal aquaculture: Reports and studies. 65th ed. Group of Experts on the Scientific Aspects ofMarine Environmental Protection, Rome. P. 54

.

Gharaei-Fathabad, E. (2011). Biosurfactants in pharmaceutical industry: A mini-review. *American Journal of Drug Discovery and Development*, **1**: 58 – 69.

Gorleku, M.A., Carboo, D., Palm, L.M.N., Quasie, W.J. and Armah, A.K. (2014). Polycyclic aromatic hydrocarbons (PAHs) pollution in marine waters and sediments at the Tema Harbour, Ghana. *Academic Journal of Environmental Science*, **2** (7): 108 – 115.

Guo, C.L., Zhou, H. W., Wong, Y. S. and Tam, N. F. Y. (2005). Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. *Marine Pollution Bulletin*, **51**: 1054 – 1061.

Gupte, A., Tripathi, A., Patel, H., Rudakiya, D. and Gupte, S. (2016).Bioremediation of Polycyclic Aromatic Hydrocarbon (PAHs): A Perspective. *Open Biotechnology Journal*,**10** (2): 363 – 378.

Hassanshahian, M., Emtiazi, G., Kermanshahi, R. K. and Cappello, S. (2010). Comparison of oil degrading microbial communities in sediments from the Persian Gulf and Caspian Sea'. *Soil and Sediment Contamination: An International Journal*, **19**: (3): 277 – 291.

Health Canada, (2014). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document–Toluene, Ethylbenzene and Xylenes. Cat. No. H144 - 20/2015E-PDF.Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Pp. 58 – 200.

Health Canada, (2016). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document - Benzo[a]pyrene. Catalogue No H144-35/2016E-PDF.Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. P. 25.

Hemalatha, S. and Veeramanikandan, P. (2011). Characterization of aromatic hydrocarbon degrading bacteria from petroleum contaminated sites. *Journal of Environmental Protection*, **2**: 243 – 254.

Hesham, A.E., Mawad, A.M.M., Mostafa, Y.M. and Shoreit, A. (2014). Biodegradation ability and catabolic genes of petroleum-degrading *Sphingomonas koreensis* strain ASU-06 isolated from Egyptian oily soil. *BioMed Research International*, **2014:** 127674.

HPA (2007). Staining procedures BSOP TP 39: National standard method, Issue 1, Standards Unit, Evaluations and Standards Laboratory Centre for Infections, Health Protection Agency, UK. Pp. 1 – 29.

Husain, S. (2008). Effect of ferric iron on siderophore production and pyrene degradation by *Pseudomonas fluorescens* 29L. *Current Microbiology*, **57**: 331 – 334.

Ichor, T., Okerentugba, P.O. and Okpokwasili, G.C. (2014). Biodegradation of total petroleum hydrocarbons by aerobic heterotrophic bacteria isolated from crude oil - contaminated brackish waters of Bodo Creek. *Journal of Bioremediation and Biodegradation,* **5** (5): 236 – 241.

Igwo-Ezikpo, M.N., Gbenle, O.G. and Ilori, M.O. (2006). Growth study on chrysene degraders isolated from polycyclic aromatic hydrocarbon polluted soils in Nigeria. *African Journal of Biotechnology*, **5**: 823 – 828.

Ijah, U.J. and Abioye, O.P. (2003). Assessment of physicochemical and microbiological properties of soil 30 months after kerosene spill. *International Journal of Research on Science and Management*,**1** (1): 24 – 30.

Irshaid, F.I. and Jacob, J.H. (2015). Screening and characterization of aerobic xylene-degrading bacteria from gasoline contaminated soil sites around gas stations in Northern Jordan. *Journal of Biological Sciences*,**15** (4): 167 – 176.

Isiodu, G.G., Stanley, H.O., Ezebuiro, V. and Okerentugba, P.O. (2016). Role of plasmid-borne genes in the biodegradation of polycyclic aromatic hydrocarbons (PAHs) by consortium of aerobic heterotrophic bacteria. *Journal of Petroleum and Environmental Biotechnology*, **7** (1): 264.

Issa, N.M. and El-Sherif, N.M. (2015) Histological and immunohistochemical study on the toxic effects of anthracene on the lung and liver of adult male albino rats and the possible protective role of *Ocimum gratissimum* extract. *Journal of Cell Biology and Histology*, **1**(1): 103.

Jacobson, G.A.S. and McLean, S. (2003). Biological monitoring of low level occupational xylene exposure and the role of recent exposure. *Annals of OccupationalHygiene*, **47** (4): 331–336.

Jaysanker, D., Ramaiah, N., Bhosle, N. B., Garg, A., Vardanyan, L., Nagle, V. L. and Fukami, K. (2007). Potential of mercury - resistant marine bacteria for detoxification of chemicals of environmental concern. *Microbes and Environ*ment, **22** (4):336 – 345.

John, R. C., Essien, J. P., Akpan, S.B. and Okpokwasili, G.C. (2012). Polycyclic aromatic hydrocarbon degrading bacteria from aviation fuel spill site at Ibeno, Nigeria. *Bulletin in Environmental Contamination and Toxicology*,**88**:1014 – 1019.

John, R.C. and Okpokwasili, G.C. (2012). Crude oil-degradation and plasmid profile of nitrifying bacteriaisolated from oil-impacted mangrove sediment in the Niger Delta of Nigeria.*Bulletin on Environmental Contamination and Toxicology*,**88**:1020 – 1026.

Jyothi, N. and Umamaheswara, R. V. (2009). Protease and urease production during utilization of diesel by fluorescent *Pseudomonas* species isolated from local soil. *Iranian Journal of Microbiology*, **1**(3): 23 – 30.

Kafilzadeh, F. and Pour, F.H. (2012).Degradation of naphthalene, phenanthrene and pyrene by *Pseudomonas* sp. and *Corynebacterium* sp. in the landfills. *International Journal of Biosciences,***2** (9): 77 – 84.

Kafilzadeh, F., Pour, F.H., Tahery, Y. and Azad, H. N. (2012).Bioremediation of pyrene by isolated bacterial strains from the soil of the landfills in Shiraz (Iran). *Annals of Biological Research***, 3** (1): 486 – 494.

Kandyala, R., Raghavendra, S.P.C. and Rajasekharan, S.T. (2010). Xylene: An overview of its health hazards and preventive measures. *Journal of Oral and Maxillofacial Pathology,* **14**(1): 1–5.

Khoshnood, R., Jaafarzadeh, N., Jamili S., Farshchi, P. and Taghavi, L. (2017). Acute toxicity of TiO, CuO and ZnO nanoparticles in brine shrimp, *Artemia franciscana.Iranian Journal of Fisheries Sciences*, **16** (4): 128 –1296.

Kim, J., Pérez-Pantoja, D., Silva-Rocha, R., Oliveros, J.C. and de Lorenzo, V. (2015).High-resolution analysis of the *m*-xylene/toluene biodegradation subtranscriptome of *Pseudomonasputida* mt-2. Society for Applied Microbiology and John Wiley & Sons Ltd, *Environmental Microbiology*, (2015): doi:10.1111/1462-2920.13054.

Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A., Wang, J., Yu, B., Zhang, J. and Bryant, S.H. (2016). PubChem Substance and Compound databases. *Nucleic Acids Research,* **44**: 1202 – 1213.

Kumar, G., Singla, R. and Kumar, R. (2010). Plasmid - associated anthracene degradation by *Pseudomonas* sp.isolated from filling station site. *Nature and Science*, **8** (4): 89.

Kwan, K. K. and Dutka, B. J. (1995). Comparative assessment of two solid - phase toxicity bioassays: The direct sediment toxicity testing procedure (DSTTP) and the Microtox_solid-phasetest (SPT). *Bullettin of Environmental Contamination and Toxicology,* **55**:338 - 346.

Lakshmi, B. M. and Velan, M (2013). Kinetics and metabolism of anthracene by *Alcaligenes faecalis* MVMB1. *International Journal of Applied Bioengineering*, **7** (2): 24 – 32.

Larsen, J.C. (2013). Polyaromatic hydrocarbons (PAH): Evaluation of health hazards and estimation of a quality criterion in soil. ISBN no. 978-87-93026-78-0. The Danish Environmental Protection Agency, Copenhagen, Denmark. Pp. 7 – 8.

Lawal, A.T. (2017). Polycyclic aromatic hydrocarbons: A review. *Cogent Environmental Science*,**3**: 1339841.

Lily, M.K., Bahuguna, A., Bhatt, K.K. and Dangwal, K. (2013). Degradation of anthracene by a novel strain *Brachybacterium paraconglomeratum* BMIT637C (MTCC 9445). *International Journal of Environmental Sciences*, **3** (4): 1242 – 1252.

Louvado, A., Gomes, N. C. M., Simões, M. M. Q., Almeida, A., Cleary, D. F. R. and Cunha, A. (2015). Polycyclic aromatic hydrocarbons in deep sea sediments: microbe–pollutant interactions in a remote environment. *Science of the Total Environment*, **526**, 312 – 328.

Mao, J., Luo, Y., Teng, Y. and Li, Z. (2012). Bioremediation of polycyclic aromatic hydrocarbon-contaminated soil by a bacterial consortium and associated microbial community changes. *International Biodeterioration and Biodegradation*, **70**: 141 – 147.

Meliani, A. and Bensoltane, A. (2014). Enhancement of hydrocarbons degradation by use of *Pseudomonas* biosurfactants and biofilms. *Journal of Petroleum and Environmental Biotechnology*, **5**: 168.

Mirdamadian S.H., Emtiazi, G., Golabi, M.H. and Ghanavati, H. (2010). Biodegradation of petroleum and aromatic hydrocarbons by bacteria isolated from petroleum-contaminated soil. *Journal of Petroleum and Environmental Biotechnology*, **1**:102.

Moorthy, B., Chu, C. and Carlin, D.J. (2015). Polycyclic aromatic hydrocarbons: From metabolism to lung cancer. *Toxicological Sciences*, **145** (1): 5 – 15.

Morasch, B., Schink, B., Tebbe, C.C. and Meckenstock, R.U. (2004). Degradation of o-xylene and m-xylene by a novel sulfate-reducer belonging to the genus *Desulfotomaculum*. *Archives of Microbiology*, **181**(6): 407 – 417.

Morcos, N.Y.S., Diab, M. R., Allah, A.A. and Borgsh**,** I. H.I.A. (2015). Acute health effects of chloroform and xylene in mice. ResearchGate, Pp. 1 – 25.

Mrozik, A., Piotrowska-Seget, Z. and Łabuzek, S. (2003). Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. *Polish Journal of Environmental Studies*, **12**: 15 – 25.

Nakamura, F.M., Germano, M.G. and Tsai, S.M. (2014). Capacity of aromatic compound degradation by bacteria from Amazon Dark Earth. *Diversity*, **6**: 339 – 353.

NNPC(1984). Monthly petroleum information (September). Nigerian National Petroleum Cooperation, Lagos, Nigeria, P. 53.

Nwinyi, O. C., Picardal, F.C., An, T.T. and Amund, O.O. (2013). Aerobic degradation of naphthalene, fluoranthene, pyrene and chrysene using indigenous strains of bacteria isolatedfrom a former industrial site. *Canadian Journal of Pure and Applied Sciences*. **7** (2): 2303 – 2314.

Obiajunwa, E. I., Pelemo, D. A., Owolabi, S. A., Fasasi, M. K. and Johnson-Fatokun, F. O. (2002). Characterisation of heavy metal pollutants of soils and sediments around a crude-oil production terminal using EDXRF. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materialsand Atoms*, **194**(1): 61 – 64.

Ogunfowokan, A. O., Okoh, E. K., Adenuga, A. A. and Asubiojo, O. I. (2005). An assessment of the impact of point source pollution from a university sewage treatment oxidation pond on a receiving stream – a preliminary study. *Journal of AppliedSciences*, **5** (1): 36 – 43.

Okani, C., Akang, E. and Ogunbiyi, O. (2013). Incidence of sub-clinical prostatic disease at autopsy in the Department of Pathology, University College Hospital, Ibadan. *Open Journal of Urology*, **4** (3): 80 – 86.

Okerentugba, P.O. and Ezeronye, O.U. (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *AfricanJournal of Biotechnology*, **2** (9):288 – 292.

Okhumode, H. Y. (2017).Addressing environmental health problems inOgoniland through implementation of UnitedNations Environment Program Recommendations:Environmental Management Strategies. *Environments,* **4** (28): 1 – 19.

Okoh, A.I. (2003). Biodegradation of Bonny light crude oil in soil microcosm by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. *African Journal of Biotechnology,* **2** (5):104 – 108.

Okoh, A.I. (2006).Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnology and Molecular Biology Review*, **1** (2): 38 – 50.

Oldersma, H., van Dijk, N.R.M. and Hanstveit, A.O. (2004). Determination of the effect of p-xylene (CAS #106 – 42 – 3) on the growth of the fresh water green alga *Selenastrum capricornutum*. TNO Report | V5819/01 | final, Netherlands Organisation for Applied Scientific Research, Netherlands Pp. 1 – 36.

Olusola, J.O. and Festus, A.A. (2015). Assessment of heavy metals in some marine fish species relevant to their concentration in water and sediment from coastal waters of Ondo State, Nigeria. *Journal of Marine Science and Research Development*, **5**: 163.

Otenio, M. H., Lopes da Silva, M. T., Marques, M. L. O., Roseiro, J.C. and Bidoia, E.D. (2005). Benzene, toluene and xylene biodegradation by *Pseudomonas putida* Ccmi 852. *Brazilian Journal of Microbiology,* **36**:258 – 261.

Owamah, H. I. (2013). Heavy metals determination and assessment in a petroleum impacted River in the Niger Delta Region of Nigeria. *Journal of Petroleum and Environmental Biotechnology*, **4**(1): 1 – 4.

Oyedele, D.J., Gasu, M.B. and Awotoye, O.O. (2008). Changes in soil properties and plant uptake of heavy metals on selected municipal solid waste dump site in Ile-Ife, Nigeria. *African Journal of Environmental Science and Technology*, **3**(5):107 – 115.

Pandey, A.K., Chaudhary, P., Singh, S.B., Arora, A., Kumar, K., Chaudhry, S. and Nain, L. (2012). Deciphering the traits associated with PAH degradation by a novel *Serratia marcescens* L-11 strain.*Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, **47** (5): 755 – 765.

Paniagua-Michel, J. and Rosales, A. (2015). Marine bioremediation - A sustainable biotechnology of petroleum hydrocarbons biodegradation in coastal and marine environments. *Journal of Bioremediation and Biodegradation*, **6**: 273.

Pathak, H. and Bhatnagar, K. (2011). *Alcaligenes*-the 4T engine oil degrader. *Journal of Bioremediation and Biodegradation*, **2** (4): 2 – 5.

Patowary, K., Kalita, M.C. and Deka, S. (2015a). Degradation of polycyclic aromatic hydrocarbons (PAHs) employing biosurfactant producing *Pseudomonas aeruginosa* KS3.*Indian Journal of Biotechnology*,**14**: 208 – 215.

Patowary, K., Saikia, R.R., Kalita, M.C. and Deka, S. (2015b). Degradation of polyaromatic hydrocarbons employing biosurfactant-producing *Bacillus pumilus* KSs2. *Annals of Microbiology*,**65**: 225 – 234.

Penezić, A., Gašparović, B., Stipaničev, D. and Nelson, A. (2014). *In-situ* electrochemical method for detecting freely dissolved polycyclic aromatic hydrocarbons in water. *Environmental Chemistry*, **11**: 173 – 180.

Peng, R.H., Xiong, A.S., Xue, Y., Fu, X.Y., Gao, F., Zhao, W., Tian, Y.S. and Yao, Q.H. (2008). Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiology Review*, **32**: 927 –955.

Perfumo, A., Banat, I.M., Marchant, R. and Vezzulli, L. (2007). Thermally enchanced approaches for bioremediation of hydrocarbon-contaminated soil. *Chemosphere*,**66** (1):179 – 184.

Poornachander, R. M., Anitha, Y. and Satyaprasad, K. (2016). Abilities of *Bacillus cereus* CPOU13 in biodegradation of polycyclic aromatic hydrocarbons (PAHs). *Journal of Pharmaceutical, Chemical and Biological Sciences*, **4** (1): 54 – 64.

Qazi, M.A., Malik, Z.A., Qureshi, G.D., Hameed, A. and Ahmed, S. (2013). Yeast extract as the most preferable substrate for optimized biosurfactant production by *rhl*B gene positive *Pseudomonas putida* SOL-10 isolate. *Journal of Bioremediation and Biodegradation*, **4** (7): 204.

Qi, Y.B., Wang, C.Y., Lv, C.Y., Lun, Z.M. and Zheng, C.G. (2017). Removal capacities of polycyclic aromatic hydrocarbons (PAHs) by a newly isolated strain from oilfield produced water. *International Journal of Environmental Research and Public Health*, **14**, (215): 1 – 12.

Rajaei, S., Seyedi, S.M., Raiesi, F., Shiran, B. and Raheb, J. (2013). Characterization and potentials of oil-degrading bacteria inhabiting the rhizosphere of Wild Oat (*Avena Fatua* L.) in South West of Iran. *Iranian Journal of Biotechnology*, **11**(1): 32 – 40.

Rajan, T. S. and Malathi, N. (2014). Health hazards of xylene: a literature review. National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA. *Journal of Clinical Diagnostic Research*, **13**: 302 – 305.

Rasool, S.A., Ijaz, S. and Subhan, S.A. (2003). Hydrocarbon degradation by marine bacteria: screening and genetic manipulation. *Pakistan Journal of Botany*, **35** (1): 37 – 44.

Rezaei-Moghadam, A., Mohajeri, D., Rafiei, B., Dizaji, R., Azhdari, A., Yeganehzad, M., Shahidi, M. and Mazani, M. (2012). Effect of turmeric and carrot seed extracts on serum liver biomarkers and hepatic lipid peroxidation, antioxidant enzymes and total antioxidant status in rats. *Bioimpacts*, **2** (3): 151 – 157.

Rodd, A.L., Creighton, M.A., Vaslet, C.A., Rangel-Mendez, J.R., Hurt, R.H. and Kane, A.B. **(**2014).Effects of surface-engineered nanoparticle-based dispersants for marine oil spills on the model organism *Artemia franciscana*. *Environmental Science and Technology*,48(11): 6419 –642.

Rohde, C. and Henze, B. (2011). Plasmid isolation from bacteria: some fast step-by-step procedures tried out at the DSMZ. DSMZ GmbH, Germany. Pp 1 – 5.

Ross, J., Nelson, G. and Erexson, G. (2002). DNA adducts in rat lung, liver and peripheral blood lymphocytes produced by oral administration of benzo[a]pyrene metabolites and derivatives. *Carcinogenesis*, **12**: 1953 – 1955.

Saillenfait, A.M., Gallissot, F., Morel, G. and Bonnet, P. (2003). Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure. *Food and Chemical Toxicology*, **41**:415 – 429.

Sarma, P.M., Bhattacharya, D., Krishnan, S. and Lal, B. (2010). Degradation of polycyclic aromatic hydrocarbons by a newly discovered enteric bacterium, *Leclercia adecarboxylata. Applied Environmental Microbiology*, **70**: 3163 – 3166.

Selivanovskaya, S.Y., Latypova, V.Z., Stepanova, N.Y. and Hung, Y.T. (2006). Bioassay of industrial waste pollutants.Cited in Waste Treatment in the Process Industries. Wang, L.K., Hung, Y.T., Lo, H.H. and Yapijakis, C. (Eds.) CRC Press Taylor & Francis Group, LCC, USA. Pp. 15 – 16.

Seo, J.S., Keum, Y.S and Li, Q. X. (2009). Bacterial degradation of aromatic compounds.*International Journal of Environmental Research and Public Health*, **6** (1): 278 – 309.

Šestinová, O., Findoráková, L. and Hančuľák, J. (2012). Toxicity testing of sediments. *Nova Biotechnologica et Chimica,* **11- 12**: 111 –116.

Shell Nigeria (2018). Oil Spill Data for 2011 – 2018. The Shell Petroleum Development Company of Nigeria Limited, Nigeria. Pp. 3 – 147.

Singh, S.N., Kumari, B., Upadhyay, S.K., Mishra, S. and Kumar, D. (2013).Bacterial degradation of pyrene in minimal salt medium mediatedby catechol dioxygenases: Enzyme purification and molecular sizedetermination. *Bioresource Technology,***133**: 293 – 300.

Srujana, K. and Khan, B. A. (2012). Isolation and characterization of polycyclic aromatic hydrocarbon degrading soil microbes from automobile workshop sediments. *Journal of Environmental Science and Technology,* **5** (1): 74 – 83.

Swaathy, S., Kavitha, V., Pravin, A.S., Mandal, A.B. and Gnanamani, A. (2014a). Microbial surfactant mediated degradation of anthracene in aqueous phase by marine *Bacillus licheniformis* MTCC 5514. *Biotechnology Reports*, **4**:161–170.

Swaathy, S., Kavitha, V., Pravin, A.S., Sekaran, G., Mandal, A.B. and Gnanamani, A. (2014b). Phylogenetic framework and biosurfactant gene expression analysis of marine *Bacillus* spp. of Eastern Coastal Plain of Tamil Nadu. *International Journal of Bacteriology,***2014**: 860491.

Taiwo, A. M., Olujimi, O.O., Bamgbose, O. and Arowolo, T.A. (2012). Surface water quality monitoring in Nigeria: Situational analysis and future management strategy, water quality monitoring and assessment, Dr. Voudouris (Ed.), ISBN: 978-953-51-0486-5, InTech, Available from: http://www.intechopen.com/books/waterquality-monitoring-and-assessment/surface-water-quality-monitoring-in-nigeria-situational-analysis-and-futuremanagement- strategy. 05/04/17.

Teh, Z. C. and Hadibarata, T. (2014). Enhanced degradation of pyrene and metabolite identification by *Pleurotus eryngii* F032. *Water Air Soil Pollution,***225**:1909.

Thavasi, R., Jayalakshmi, S., Balasubramanian, T and Banat, I.M. (2006). Biodegradation of crude oil by nitrogen fixing marine bacterium *Azotobacter chroococcum. Research Journal of Microbiology*,**1** (5): 401 – 408.

Twumasi, Y. and Merem, E. (2006). GIS and remote sensing applications in the assessment of change within a coastal environment in the Niger Delta region of Nigeria. *International Journal ofEnvironmental Research and Public Health*, **3** (1):98 – 106.

Ubani, O., Atagana, H.I., Thantsha, M.S. and Rasheed, A. (2016). Identification and characterisation of oil sludge degrading bacteria isolated from compost. *Archives of Environmental Protection*,**42** (2): 67–77.

UNDP (2006). Niger Delta Development Human Report. United Nations Development Report, New York. Cited in P. B. Eregha and I. R. Irughe, (2009). Oil induced environmental degradation in the Nigeria's Niger Delta: The Multiplier Effects. *Journal of Sustainable Development in Africa*,**11** (4):162 – 163.

UNEP(2011). Environmental assessment of Ogoniland.United Nations Environment Programme, Nairobi, Kenya. Pp. 9 – 11.

Vainer, B., Toft, B. G., Olsen, K. E., Jacobseen, G. K. and Marcussen, N. (2011). Handling of radical prostatectomy specimens: Total or partial Embedding. *Histopathology*, **58** (2): 211 – 216.

Vijayakumar, S. and Saravanan, V. (2015). Biosurfactants-types, sources and applications. *Research Journal of Microbiology*, **10**(5): 181 – 192.

Vyas, T. K. and Dave, B. P. (2011). Production of biosurfactant by *Nocardia otitidiscaviarum* and its role in biodegradation of crude oil. *International Journal of Environmental Science and Technology*, **8** (2): 425 – 432.

Wang, Q. and Xue, Y. (2015). Characterization of solid tumors induced by polycyclic aromatic hydrocarbons in mice. *Medical Science Monitoring Basic Research,* **21**: 81 – 85.

Wanjohi, L., Mwamburi, L., Too, E., Aloo, B. and Kosgei, J. (2015). Isolation and identification of bacteria with bioremediation potential of oil spills in lake Nakuru, Kenya. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, **17** (4): 831 – 838.

WHO (2003). Polynuclear aromatic hydrocarbons in drinking water: Background document for development ofWHO guidelines for drinking water quality. WHO/SDE/WSH/03.04/59, World Health Organization, Geneva, Switzerland. Pp. 4 – 10.

WHO (2008). Guidelines for Drinking Water Quality. 3rd Vol. World Health Organization, Geneva, Switzerland. Pp. 156 – 194.

Willey, J. M., Sherwood, I. M. and Woolverton, C. J. (2008). Prescott, Harley and Kleins Microbiology, 7th ed. McGraw-Hill companies, New York. Pp. 537 – 813.

`

Xu, R., Lau, N.L.A., Ng, K.L. and Obbard, J.P. (2004). Application of a slow-release fertilizer for oil bioremediation in beach sediments. *Journal of Environmental Quality,***33**: 1210 – 1216.

Yuliani, H., Sahlan, M., Hermansyah, H. and Wijanarko, A. (2012).Selection and identificationof polyaromatic hydrocarbon degrading bacteria. *World Applied Sciences Journal*, **20** (8): 1133 – 1138.

APPENDIX

Appendix I: Features of the three sampling sites

The features of the three sampling sites are shown in Appendix I. The sampled points parameters of Abonema, Nembe and Onne are presented on Appendices Ia - c.

The photographs and thematic maps indicating the locations of sample points and its landcover in Abonema, Nembe and Onne studied areas are shownAppendices Id – i.

Appendix Ia: Abonema sampled points parameters

Sampled points	Latitude (D)	Longitude (D)	Earthing (m)	Northing (M)	Elevation (M)
1	4°46'29.72"N	7° 0'17.20"E	278703.02 m E	528103.91 m N	4
2	4°46'30.74"N	7° 0'11.85"E	278538.16 m E	528134.52 m N	5
3	4°46'38.01"N	7° 0'9.80"E	278474.51 m E	528358.94 m N	4
4	4°46'35.83"N	7° 0'1.51"E	278219.24 m E	528288.96 m N	4
5	4°46'26.16"N	7° 0'0.54"E	278187.58 m E	527994.98 m N	2
6	4°46'24.07"N	7° 0'5.35"E	278335.88 m E	527932.71 m N	1
7	4°46'22.72"N	7° 0'18.27"E	278733.91 m E	527888.91 m N	5
8	4°46'24.65"N	7° 0'31.87"E	279152.57 m E	527942.57 m N	7
9	4°46'18.17"N	7° 0'34.82"E	279243.79 m E	527746.44 m N	6
10	4°46'15.82"N	7° 0'25.15"E	278945.21 m E	527671.47 m N	3

Appendix Ib: Nembe sampled points parameters

Sampled points	Latitude (D)	Longitude (D)	Earthing (m)	Northing (M)	Elevation (M)
1	4°45'26.42"N	7° 1'38.84"E	281213.07 m E	526151.91 m N	2
2	4°45'23.28"N	7° 1'46.72"E	281453.65 m E	526052.80 m N	1
3	4°45'19.62"N	7° 1'51.09"E	281583.85 m E	525936.17 m N	1
4	4°45'15.87"N	7° 2'2.64"E	281947.23 m E	525827.97 m N	5
5	4°45'8.72"N	7° 2'14.54"E	282313.94 m E	525606.33 m N	7
6	4°45'10.29"N	7° 1'54.76"E	281540.77 m E	525799.86 m N	1
7	4°45'10.03"N	7° 1'54.76"E	281700.21 m E	525645.08 m N	1
8	4°45'19.01"N	7° 1'32.37"E	281010.48 m E	525922.15 m N	4
9	4°45'15.69"N	7° 1'11.37"E	280365.00 m E	525825.78 m N	0
10	4°45'25.98"N	7° 1'22.57"E	280710.78 m E	526134.99 m N	5

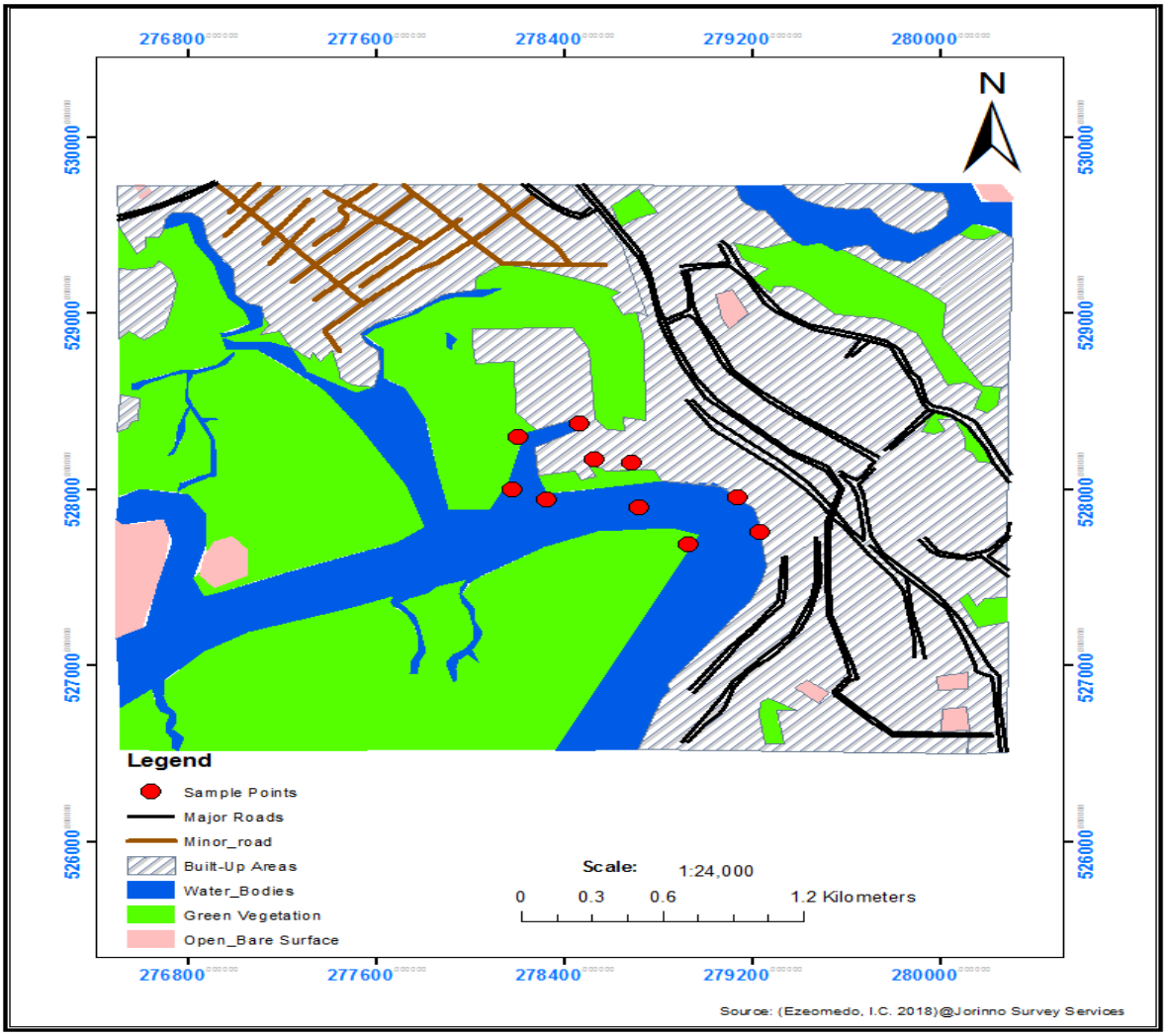
Appendix Ic: Onne sampled points parameters

Sampled points	Latitude (D)	Longitude (D)	Earthing (m)	Northing (M)	Elevation (M)
1	4°41'43.29"N	7°10'48.82"E	298147.49 m E	519246.40 m N	1
2	4°41'40.37"N	7°10'43.39"E	297975.75 m E	519161.80 m N	2
3	4°41'32.58"N	7°10'28.13"E	297505.57 m E	518927.22 m N	1
4	4°41'32.83"N	7°10'10.64"E	296969.67 m E	518931.05 m N	1
5	4°41'33.59"N	7° 9'54.17"E	296460.06 m E	518957.42 m N	5
6	4°41'43.85"N	7° 9'27.59"E	295640.62 m E	519274.98 m N	2
7	4°41'58.18"N	7° 9'26.34"E	295603.49 m E	519714.30 m N	2
8	4°41'43.93"N	7° 9'51.68"E	296382.49 m E	519276.49 m N	3
9	4°41'40.41"N	7°10'10.49"E	296965.14 m E	519165.67 m N	3
10	4°41'49.13"N	7°10'41.18"E	297915.42 m E	519432.72 m N	3

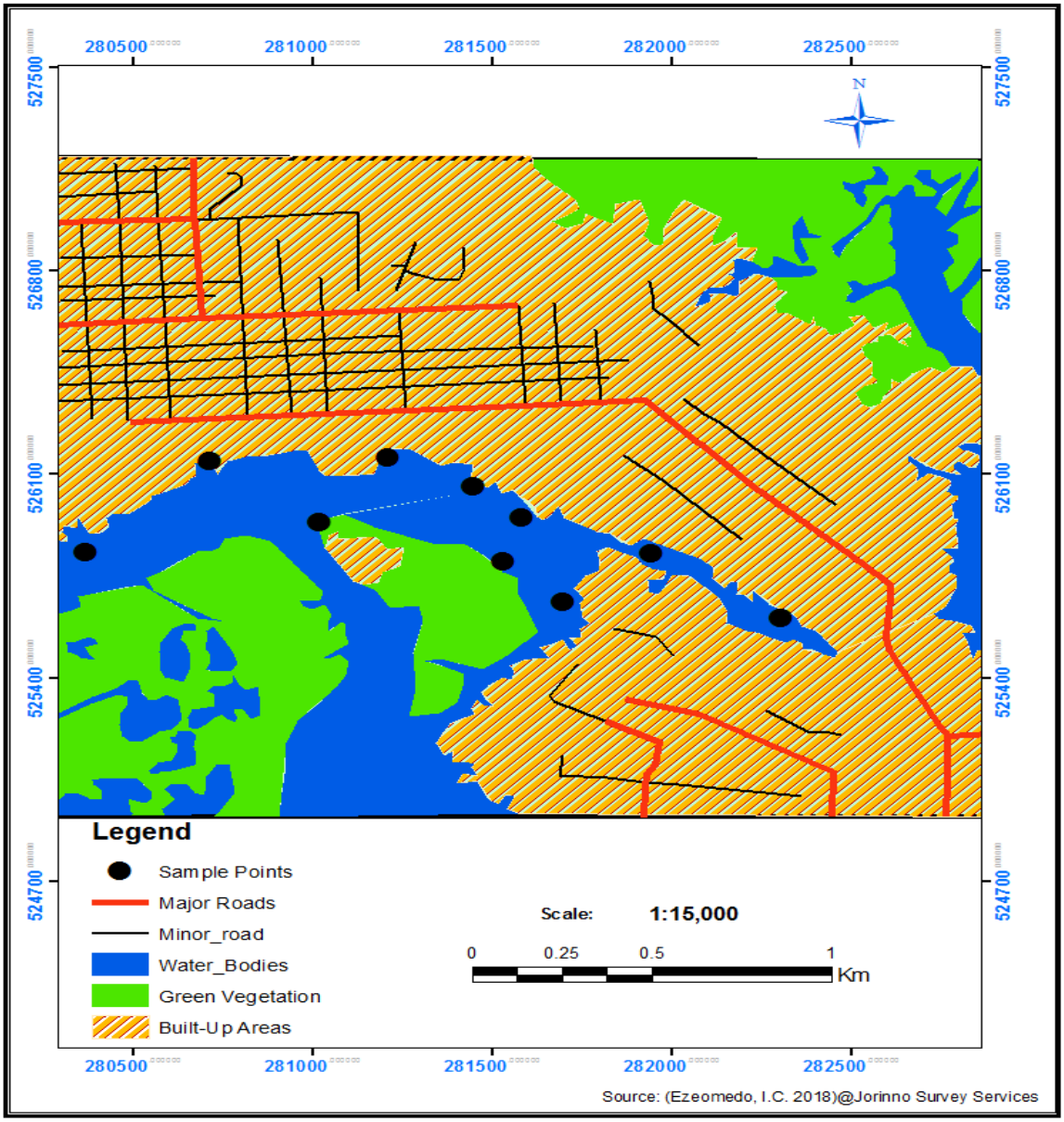
Appendix Id: Abonema Wharf Water Front sampling site as photographed by the researcher

Appendix Ie: Nembe Water-Side sampling siteas photographed by the researcher

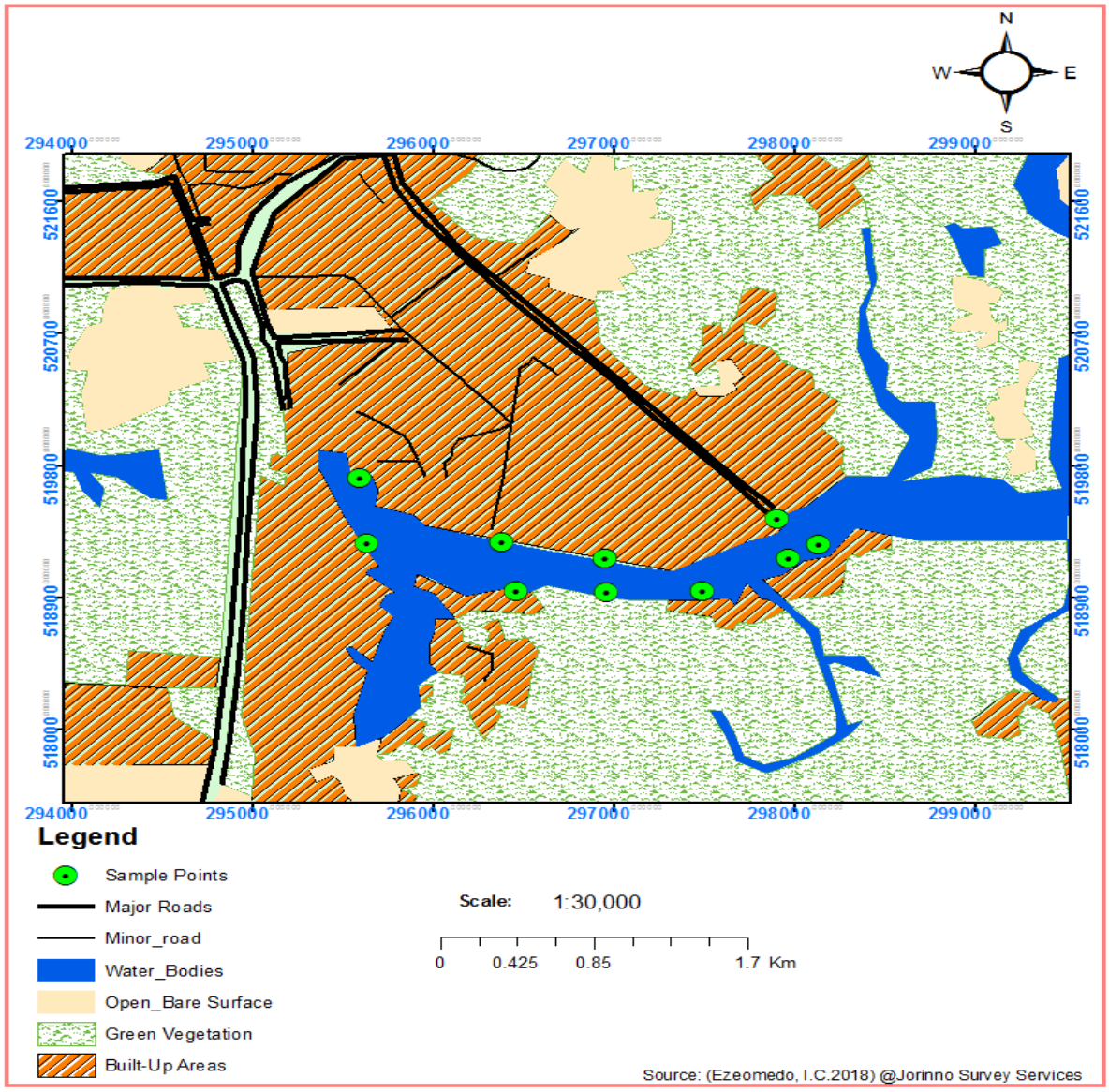
Appendix If: Onne Light Terminal Flow sampling site as photographed by the researcher



Appendix Ig: Thematic map indicating the locations of sample points and its landcover in Abonema study area



Appendix Ih: Thematic map indicating the locations of sample points and its landcover in Nembe study area



Appendix Ii: Thematic map indicating the locations of sample points and its landcover in Onne study area

Appendix II: Toxicity Profile

The results of Mean values of cell algal density measurements (cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of xylene, anthracene, pyrene, contaminated sediment samples andfeatures of the marine toxicity test with microalgaare presented in Appendices IIa – e.

The results of Germination index (GI) of *Sinapsis alba* (mustard seeds) grown on the aromatic hydrocarbon contaminated sediment samples and positive control (250 mg/kg/l) and test concept of the phytotoxicity are presented in Appendices IIf – g.

The results of the percentage mortality scores of *Artemia franciscana* (brine shrimp) exposed to aromatic hydrocarbon contaminated samples and features of the *Artemia* toxicity screening test are presented in Appendices IIh – i.

The results of the absorbance values of the toxicity of the aromatic hydrocarbons in distiiled water and wastewater of the three sampled locations using Toxi -chromotest at different dilutions and microplate test result for Toxi - chromo testing of aromatic hydrocarbon compounds are presented in AppendicesIIj- k.

Appendix IIa: Mean values of cell algal density measurements (cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of xylene

Parameters	Concentration					
	0 mg/l	1.0 mg/l	1.8 mg/l	3.2 mg/l	5.6 mg/l	18 mg/l
Time (h)	Xylene + Distilled water					
0	70.00	52.50	49.00	28.00	28.00	26.00
24	160.00	28.00	26.00	20.00	12.00	49.00
48	265.00	20.00	20.00	12.00	6.25	12.00
72	625.00	12.00	12.00	6.25	6.25	1.00
CV (%)	86.93	62.27	59.45	57.26	78.33	94.11
Specific growth rate	8.30	3.30	1.60	1.30	1.00	0.80
% Inhibition	1.33	30.24	50.72	54.34	57.95	60.36
Time (h)	Xylene + Abonema sediment					
0	70.00	125.00	61.50	49.00	27.00	20.00
24	160.00	72.00	52.50	47.00	22.00	82.50
48	265.00	26.50	30.00	22.00	14.00	6.25
72	625.00	14.00	14.00	8.25	6.25	2.25
CV (%)	86.93	84.80	54.56	62.76	52.64	134.35
Specific growth rate	8.30	1.80	1.40	1.00	0.70	0.40
% Inhibition	1.33	78.31	83.13	87.95	91.57	95.18
Time (h)	Xylene + Nembe sediment					
0	70.00	40.00	20.00	13.00	10.00	6.25
24	160.00	30.00	13.00	8.25	8.25	6.25
48	265.00	22.50	8.50	6.25	6.25	1.00
72	625.00	8.40	6.25	6.25	6.25	1.00
CV (%)	86.93	52.77	50.79	37.74	23.51	83.62
Specific growth rate	8.30	3.30	1.80	1.10	0.80	0.40
% Inhibition	1.33	60.20	78.31	86.75	90.36	95.18
Time (h)	Xylene + Onne sediment					
0	70.00	26.00	24.00	20.00	14.00	6.25
24	160.00	26.00	20.00	85.00	8.25	6.25
48	265.00	15.00	8.50	6.25	6.25	6.25
72	625.00	1.00	6.25	6.25	6.25	1.00
CV (%)	86.93	69.77	58.89	128.16	42.19	53.16
Specific growth rate	8.30	1.70	1.50	1.10	0.90	0.50
% Inhibition	1.33	79.52	81.93	86.75	89.16	93.97

Appendix IIb: Mean values of cell algal density measurements (cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of anthracene

Parameters	Concentration					
	0 mg/l	1.0 mg/l	1.8 mg/l	3.2 mg/l	5.6 mg/l	18 mg/l
Time (h)	Anthracene + Distilled water					
0	71.00	265.00	22.00	22.00	13.00	13.00
24	164.00	262.00	22.00	13.00	6.25	6.25
48	268.00	250.00	6.25	6.25	1.00	0.90
72	626.00	6.25	1.00	1.00	0.90	0.90
CV (%)	86.05	64.62	84.47	85.8	108.12	109.11
Specific growth rate	8.40	2.90	1.70	1.30	0.90	0.50
% Inhibition	1.34	40.06	54.52	59.34	64.16	66.57
Time (h)	Anthracene + Abonema sediment					
0	71.00	32.00	22.00	13.00	6.25	8.25
24	164.00	30.00	50.00	8.50	1.00	6.25
48	268.00	15.00	6.25	6.25	1.00	1.00
72	626.00	6.25	6.25	6.25	1.00	1.00
CV (%)	86.05	59.20	97.67	37.44	113.51	89.69
Specific growth rate	8.40	1.90	1.50	1.00	0.60	0.30
% Inhibition	1.34	77.11	81.93	87.97	92.77	95.39
Time (h)	Anthracene + Nembe sediment					
0	71.00	32.00	24.00	20.00	13.00	10.00
24	164.00	15.00	13.00	10.00	8.00	6.25
48	268.00	8.50	6.25	6.25	6.25	1.00
72	626.00	6.25	6.25	6.25	6.25	0.00
CV (%)	86.05	75.45	67.70	61.13	38.11	108.49
Specific growth rate	8.40	2.10	1.50	1.20	0.80	0.30
% Inhibition	1.34	74.70	81.93	85.54	93.60	96.39
Time (h)	Anthracene + Onne sediment					
0	71.00	32.00	30.00	24.00	14.00	14.00
24	164.00	20.00	14.00	8.25	6.25	6.25
48	268.00	8.50	8.25	6.25	6.25	6.25
72	626.00	6.25	6.25	6.25	6.25	0.01
CV (%)	86.05	71.02	73.60	76.81	47.33	86.43
Specific growth rate	8.40	1.90	1.40	1.00	0.70	0.30
% Inhibition	1.34	77.11	83.13	87.95	91.57	96.39

Appendix IIc: Mean values of cell algal density measurements (cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of pyrene

Parameters		Concentration				
	0 mg/l	1.0 mg/l	1.8 mg/l	3.2 mg/l	5.6 mg/l	18 mg/l
Time (h)		Pyrene + Distilled water				
0	69.00	26.00	13.00	13.00	6.25	6.25
24	159.00	13.00	6.25	6.25	6.25	6.25
48	269.00	6.25	6.25	6.25	1.00	1.00
72	627.00	1.00	1.00	1.00	0.80	0.01
CV (%)	87.09	93.46	74.24	74.24	86.43	98.93
Specific growth rate	8.20	2.50	1.50	1.10	0.90	0.60
% Inhibition	1.31	49.88	61.93	66.75	69.16	72.77
Time (h)		Pyrene + Abonema sediment				
0	69.00	63.00	52.00	32.00	20.00	14.00
24	159.00	54.00	30.00	22.50	13.00	6.25
48	269.00	30.00	24.00	1.00	6.25	6.25
72	627.00	13.00	10.00	6.25	6.25	0.04
CV (%)	87.09	56.90	60.25	92.90	57.77	86.16
Specific growth rate	8.20	1.70	1.30	1.00	0.60	0.30
% Inhibition	1.31	80.72	84.34	87.95	92.77	96.39
Time (h)		Pyrene + Nembe sediment				
0	69.00	27.00	24.00	20.00	14.00	13.00
24	159.00	24.00	18.50	8.50	8.25	8.25
48	269.00	15.00	8.40	8.25	6.25	6.25
72	627.00	8.25	6.25	6.25	6.25	0.01
CV (%)	87.09	46.11	58.75	58.12	42.19	78.27
Specific growth rate	8.20	1.60	1.40	0.80	0.50	0.20
% Inhibition	1.31	80.72	83.13	90.36	93.97	98.19
Time (h)		Pyrene + Onne sediment				
0	69.00	26.00	24.00	24.00	14.00	13.00
24	159.00	22.50	14.00	14.00	13.00	13.00
48	269.00	1.00	8.25	6.25	6.25	6.25
72	627.00	6.25	6.25	6.25	6.25	0.03
CV (%)	87.09	87.41	60.64	66.67	42.59	77.24
Specific growth rate	8.20	1.50	1.20	0.80	0.50	0.10
% Inhibition	1.31	81.93	85.54	90.36	93.97	97.59

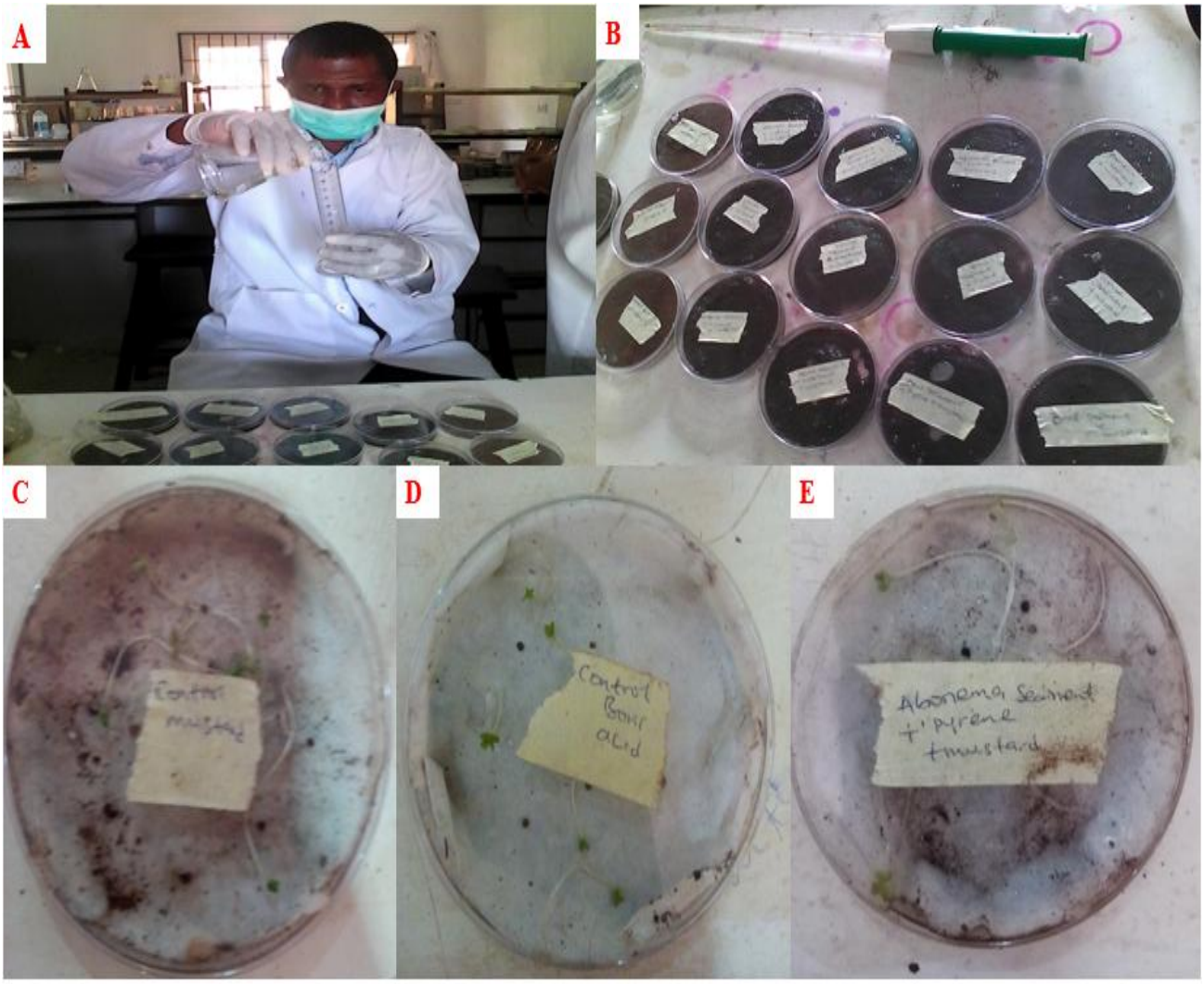
Appendix IId: Mean values of cell algal density measurements (cells/ml X 10^4) with their respective coefficient of variation, specific growth rates and percentage inhibition at different concentrations of contaminated sediment samples

Parameters	Concentration					
	0 mg/l	1.0 mg/l	1.8 mg/l	3.2 mg/l	5.6 mg/l	18 mg/l
Time (h)	Positive sample ($K_2Cr_2O_7$)					
0	72.00	262.00	60.00	49.00	28.00	26.00
24	162.00	259.00	49.00	26.00	26.00	22.00
48	267.00	252.00	26.00	28.00	22.00	6.25
72	628.00	22.00	13.00	13.00	6.25	6.25
CV (%)	86.41	59.32	57.76	51.38	47.96	68.61
Specific growth rate	8.10	1.60	1.40	1.10	1.0	0.40
% Inhibition	1.29	35.72	38.13	41.75	45.36	50.18
Time (h)	Abonema sediment					
0	72.00	220.00	185.00	26.00	15.00	28.00
24	162.00	185.00	165.00	15.00	6.25	20.00
48	267.00	130.00	87.50	6.25	6.25	6.25
72	628.00	1.00	70.00	6.25	5.00	4.00
CV (%)	86.41	71.71	44.63	70.08	56.87	78.38
Specific growth rate	8.10	1.30	1.10	1.00	1.00	0.75
% Inhibition	1.29	56.75	57.95	57.95	58.77	60.95
Time (h)	Nembe sediment					
0	72.00	100.00	82.50	50.00	52.50	60.00
24	162.00	70.00	62.50	12.00	28.00	26.00
48	267.00	52.50	49.00	12.00	12.00	13.00
72	628.00	15.00	12.00	6.25	2.00	1.00
CV (%)	86.41	59.78	57.69	100.39	93.24	101.88
Specific growth rate	8.10	2.00	1.60	1.00	0.70	0.50
% Inhibition	1.29	45.9	50.72	57.95	61.57	63.97
Time (h)	Onne sediment					
0	72.00	300.00	165.00	82.00	82.50	52.50
24	162.00	175.00	62.50	60.50	52.50	28.00
48	267.00	82.50	49.00	26.00	20.00	12.00
72	628.00	28.00	20.00	13.00	6.25	3.25
CV (%)	86.41	81.33	85.16	69.62	84.74	90.34
Specific growth rate	8.10	1.30	1.10	1.00	1.00	0.60
% Inhibition	1.29	54.34	56.75	57.95	57.95	62.77

Appendix IIe: Features of the marine toxicity test with microalga A. Contents of the Algaltoxkit. B. Preculturing of the alga. C. Preparation of samples through membrane filtration D. Transfer of the algal – toxicant dilutions into the test vials.

Appendix IIf: Germination index (GI) of *Sinapsis alba*(mustard seeds) grown on the aromatic hydrocarbon - contaminated sediment samples and positive control (250 mg/kg/l)

Sample (mg/kg/l)	Mean root length (mm)	Mean number of germinated seeds	Germination index (%)	Coefficient of variation (%)
Negative control	20.00 ± 10.50	08.00 ± 1.00	-	-
Positive control (H_3BO_3)	03.00 ± 0.02	04.00 ± 0.00	14.26 ± 0.03	0.21
Xylene + Distilled water	03.00 ± 0.10	08.00 ± 1.00	28.57 ± 0.05	0.18
Xylene + Abonema sediment	03.00 ± 0.20	07.00 ± 0.00	25.00 ± 0.20	0.83
Xylene + Nembe sediment	02.50 ± 0.30	07.00 ± 1.00	20.83 ± 0.20	0.10
Xylene + Onne sediment	03.38 ± 0.25	06.00 ± 0.00	24.14 ± 6.33	31.03
Anthracene + Distilled water	03.64 ± 0.20	06.00 ± 0.00	26.57 ± 0.04	0.15
Anthracene + Abonema sediment	03.30 ± 0.30	5.00 ± 1.00	19.64 ± 0.05	0.28
Anthracene + Nembe sediment	03.22 ± 0.10	04.00 ± 0.00	15.33 ± 0.01	0.10
Anthracene + Onne sediment	03.00 ± 0.10	05.00 ± 1.00	17.86 ± 0.08	0.45
Pyrene + Distilled water	03.13 ± 0.16	06.00 ± 2.00	22.36 ± 0.18	0.83
Pyrene + Abonema sediment	02.30 ± 0.12	04.00 ± 0.00	10.95 ± 0.47	04.53
Pyrene + Nembe sediment	02.50 ± 0.10	03.00 ± 2.00	08.90 ± 0.05	0.56
Pyrene + Onne sediment	01.00 ± 0.10	06.00 ± 1.00	07.14 ± 0.02	0.37
Abonema sediment	04.60 ± 0.20	05.00 ± 1.00	27.38 ± 0.19	0.70
Nembe sediment	05.31 ± 0.13	04.00 ± 0.00	25.28 ± 0.20	0.80
Onne sediment	03.00 ± 0.10	07.00 ± 1.00	25.00 ± 0.36	01.45



Appendix IIg: Test concept of the phytotoxicity A. Preparation of samples dilutions B. Petri dishes containing soil for seed inoculation C. Petri dish with grown control mustard seeds D. Petri dish with grown positive control boric acid mustard seeds E. Petri dish with grown Abonema sediment + pyrene mustard seeds

Appendix IIh: Percentage mortality scores of *Artemia franciscana* (brine shrimp) exposed to aromatic hydrocarbon - contaminated samples

Samples	0 mg/l	10 mg/l	18 mg/l	32 mg/l	56 mg/l	100 mg/l
Positive control ($K_2Cr_2O_7$)	06.67	20.00	23.33	30.00	46.67	66.67
Xylene + Distilled water	03.33	20.00	33.33	40.00	56.67	66.67
Xylene + Abonema water	0.00	23.33	33.33	46.67	60.00	70.00
Xylene + Nembe water	06.67	23.33	33.33	43.33	60.00	73.33
Xylene + Onne water	0.00	16.67	30.00	46.67	60.00	73.33
Anthracene + Distilled water	06.67	36.67	43.33	53.33	56.67	66.67
Anthracene + Abonema water	06.67	33.33	40.00	53.33	63.33	70.00
Anthracene + Nembe water	03.33	30.00	46.67	50.00	56.67	76.67
Anthracene + Onne water	0.00	30.00	43.33	53.33	63.33	80.00
Pyrene + Distilled water	06.67	30.00	33.33	56.67	66.67	70.00
Pyrene + Abonema water	03.33	26.67	33.33	56.67	63.33	83.33
Pyrene + Nembe water	03.33	23.33	33.33	53.33	66.67	86.67
Pyrene + Onne water	06.67	20.00	33.33	53.33	70.00	93.33
Abonema water	03.33	26.67	30.00	40.00	46.67	60.00
Nembe water	03.33	30.00	36.67	43.33	50.00	63.33
Onne water	06.67	20.00	33.33	43.33	46.67	66.67

Appendix IIi: Features of the *Artemia* toxicity screening test A. Contents of the Artoxkit M. B. Petri dish with Hatched instar II - III larvae. C. Viewing and counting of the larvae under dissecting microscope

Appendix IIj: Absorbance values of the toxicity of the aromatic hydrocarbons in distiiled water and wastewater of the three sampled locations using Toxi-chromotest at different dilutions

Samples	Positive Control	Negativ control	Xylene+ Distillec water	Xylene + Abonema water	Xylene - Nembe water	Xylene Onne water	Anthracene Distilled water	Anthracene Abonema water	Anthracene Nembe water	Anthracene Onne water	Pyrene + Distilled water	Pyrene + Abonema water	Pyrene + Nembe Water	Pyrene Onne water	Abonem water	Nembe water	On wat
Undilutec	0.146	0.156	0.142	0.121	0.146	0.159	0.155	0.188	0.183	0.294	**0.398**	0.332	0.268	0.167	0.270	0.183	0.2
½	0.127	0.156	0.122	0.146	0.128	0.135	0.155	0.236	0.150	0.235	0.240	0.330	0.230	0.178	0.258	0.190	0.2
¼	0.135	0.138	0.115	0.123	0.134	0.107	0.115	0.216	0.235	0.197	0.271	0.274	0.213	0.186	0.229	0.204	0.2
1/8	0.138	0.128	0.142	0.123	0.124	0.112	0.163	0.199	0.217	0.190	0.220	0.256	0.273	0.192	0.234	0.223	0.2
1/16	0.120	0.138	0.127	0.123	0.112	0.123	0.171	0.243	0.186	0.179	0.250	0.254	0.197	0.183	0.236	0.183	0.2
1/32	0.127	0.128	0.123	0.123	0.118	0.117	0.145	0.217	0.152	0.190	0.243	0.234	0.182	0.188	0.225	0.169	0.2
1/64	0.117	0.127	0.126	0.120	0.113	0.104	0.194	0.233	0.165	0.219	0.265	0.224	0.287	0.192	0.260	0.219	0.2
1/128			0.199	0.126	0.138	0.146	0.101	0.173	0.184	0.230	0.235	0.234	0.200	0.184	0.226	0.143	0.2
1/256			0.123	0.123	0.123	0.165	0.155	0.177	0.169	0.185	0.174	0.218	0.315	0.137	0.242	0.170	0.2
1/512			0.108	0.105	0.132	0.133	0.176	0.211	0.167	0.177	0.178	0.174	0.226	0.119	0.199	0.308	0.1
1/1024			0.113	0.111	0.138	0.161	0.156	0.182	0.155	0.155	0.218	0.185	0.276	0.144	0.289	0.196	0.3
1/2048			**0.097**	0.119	0.126	0.152	0.136	0.188	0.174	0.190	0.236	0.234	0.226	0.141	0.214	0.244	0.2
1/4096			0.123	0.112	0.131	0.153	0.175	0.189	0.166	0.181	0.259	0.208	0.308	0.161	0.308	0.238	0.2
Sample Blank			0.119	0.133	0.123	0.141	0.877	0.189	0.171	0.121	0.318	0.184	0.244	0.202	0.280	0.228	0.2
Blank		0.186	0.1755	0.195	0.2205	0.230	0.2325	0.259	0.228	0.252	0.306	0.5705	0.286	0.220	0.297	0.250	0.5

Appendix IIk: Microplate test result for Toxi - chromo testing of aromatic hydrocarbon compounds

Appendix III: Degradability Profile

The results of the aromatic hydrocarbon-degradability of marine bacterial isolates from Abonema, Nembe and Onne sampled locations and aromatic hydrocarbon utilizing bacteria isolated on mineral basal agar plates are presented in Appendices IIIa – d.

Isolate	Aromatic hydrocarbon		
	Xylene	Anthracene	Pyrene
AB1	++	+	+++
AB2	+++	+++	+++
AB3	+++	+	++
AB4	+++	++	+++
AB5	++	++	+++
AB6	++	+++	++
AB7	++	++	+++
AB8	++	+++	+++
AB9	++	++	++
AB10	+++	++	+++
AB11	+++	++	++
AB12	++	++	++
AB13	+	++	+

Appendix IIIa: Aromatic hydrocarbon-degradability of marine bacterial isolates from Abonema sampled location

KEY: Heavy growth = +++; Moderate growth = ++; Weak growth = +

Appendix IIIb: Aromatic hydrocarbon-degradability of marine bacterial isolates from Nembe sampled location

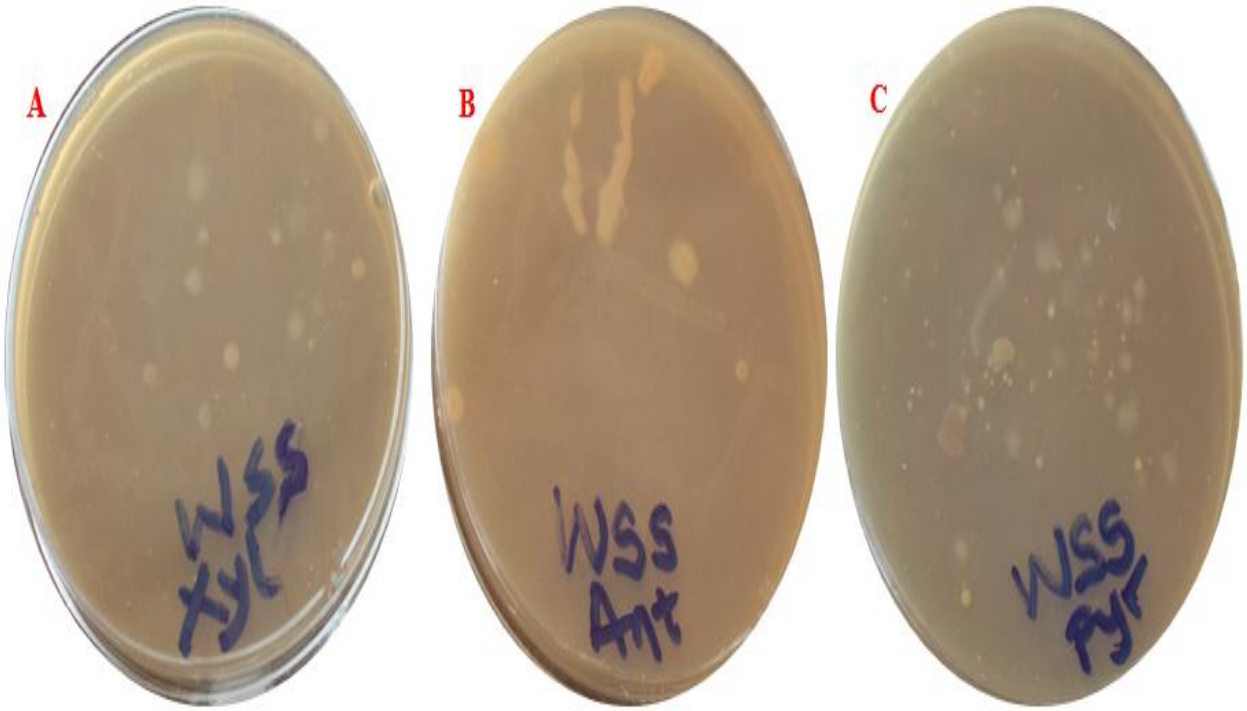
Isolate	Aromatic hydrocarbon		
	Xylene	Anthracene	Pyrene
NW1	+++	+	+++
NW2	+++	+	+++
NW3	++	+	+++
NW4	+++	+	+++
NW5	+++	+	+++
NW6	++	+	+++
NW7	++	+	+++
NW8	++	+	+++
NW9	+++	++	+++
NW10	+++	+++	+++
NW11	+++	++	+++
NW12	++	++	+++
NW13	++	+++	+++
NW14	+++	++	+++
NW15	+++	+	+++
NW16	+++	++	+++
NW17	+++	+++	++

KEY: Heavy growth = +++; Moderate growth = ++; Weak growth = +

Appendix IIIc: Aromatic hydrocarbon-degradability of marine bacterial isolates from Onne sampled location

Isolate	Aromatic hydrocarbon		
	Xylene	Anthracene	Pyrene
ON1	+++	+++	++
ON2	++	+++	++
ON3	+++	++	++
ON4	+++	+	++
ON5	++	++	++
ON6	++	++	++
ON7	++	+	+++
ON8	+	+	++
ON9	++	++	++
ON10	+	++	+++
ON11	+++	+++	++
ON12	+++	++	++
ON13	++	+	++
ON14	++	+	++
ON15	+++	++	++
ON16	+++	++	++
ON17	++	+++	++
ON18	+++	+++	++

KEY: Heavy growth = +++; Moderate growth = ++; Weak growth = +



Appendix IIId: Aromatic hydrocarbon - utilizing bacteria isolated on mineral basal agar plates A. Xylene utilizing bacteria growing on mineral basal agar plate B. Anthracene utilizing bacteria growing on mineral basal agar C. Pyrene utilizing bacteria growing on mineral basal agar

Appendix IV: Sequencing Products

The result of the aligned gene sequences details of the PCR amplified products respective to ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 genes are presented in Appendices IVa – i.

CGCGCACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCCGCACCTGAG

CGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCACTCTCTACG

CATTTCACCGCTACACATGGAATTCTCCCCCCCTCTACAAGACTCTAGCTGACCA

GTCTTAGATGCCATTCCCAGGTAAGCCCGGGGATTTCACATCTAACTTAATCAAC

CGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATT

ACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGTCGGTAACGTCAATC

GT

Appendix IVa: Aligned gene sequences details of the PCR amplified product respective to ANT1 gene

GAGGGGTCACTTTCACGCGTTAGCTGCGCTACTAAGGCCTAACGGCCCCAACAG

CTAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC

CCACGCTTTCGTGTCTGAGCGTCAGTATTATCCCAGGGGGCTGCCTTCGCCATCG

GTATTCCTCCACATATCTACGCATTTCACTGCTACACGTGGAATTCTACCCCCCTC

TGACATACTCTAGCTCGGCAGTTAAAAATGCAGTTCCAAGGTTGAGCCCTGGGAT

TTCACATCTTTCTTTCCGAACCGCCTACACACGCTTTACGCCCAGTAATTCCGATT

AACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTT

ATTCTGCAGATACCGTCAGCAGCATCCCGTATTAGGGGATACCTTTTCTTCTCTGC

CAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATC

AGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGG

CCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTT

GCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAAT

AGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGC

CACTCTTTCGAGTAGTTATCCCCCCGCTACTGGGCACGTTCCGATATATTACTCAC

CCGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTG

CATGTGTAAAGCATCCCGCTAGCGTTCAATCTGAAGTCAGGATTAAAACTCTAAC

C

Appendix IVb: Aligned gene sequences details of the PCR amplified product respective to XYL2 gene

CCCCGGGTTGCTTAGTGCGTTAGCTAGCGTCACCGAACTGCATGCAGCCCGACAA

CTAGCCATCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC

CACGCTTTCGCGCCTCAGCGTCAGTAATGAGCCAGTATGTCGCCTTCGCCACTGG

TGTTCTTCCGAATATCTACGAATTTCACCTCTACACTCGGAGTTCCACATACCTCT

CTCATACTCAAGATCGCCAGTATCAAAGGCAGTTCCAGGGTTGAGCCCTGGGATT

TCACCTCTGACTTAACGATCCGCCTACGCGCCCTTTACGCCCAGTAATTCCGAGC

AACGCTAGCCCCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTT

CTTCTCCGGGTACCGTCATTATCGTCCCCGGTGAAAGAATTTTACAATCCTAAGA

CCTTCATCATTCACGCGGCATGGCTGCGTCAGGCTTTCGCCCATTGCGCAAGATT

CCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTG

GTCATCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCCGTTACCTCACCA

ACTAGCTAATCAGACGCGGGCCGCTCTAAAGGCGATAAATCTTTCCCCCGAAGG

GCACATTCGGCATTACCACCCGTTTCCAGGAGCTATTCCGAACCTAAAGGCACGT

TCCCACGTGTTACTCACCCGTCCGCCACTAACCCCGAAGGGTCCGTTCGACTTGC

ATGTGTTAGGCCTGCCGCCAGCGTTCGCTCTGAGCCAGGAATCAAACTAAG

Appendix IVc: Aligned gene sequences details of the PCR amplified product respective to PYR3 gene

CGGGTCAACTTCACGCGTTAGCTGCGCTACTAAGGCCTAACGGCCCCAACAGCTA

GTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCA

CGCTTTCGTGTCTGAGCGTCAGTATTATCCCAGGGGGCTGCCTTCGCCATCGGTA

TTCCTCCACATATCTACGCATTTCACTGCTACACGTGGAATTCTACCCCCCTCTGA

CATACTCTAGCTCGGCAGTTAAAAATGCAGTTCCAAGGTTGAGCCCTGGGATTTC

ACATCTTTCTTTCCGAACCGCCTACACACGCTTTACGCCCAGTAATTCCGATTAAC

GCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATT

CTGCAGATACCGTCAGCAGTATCTCGTATTAGGAGATACCTTTTCTTCTCTGCCAA

AAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGG

GTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCG

TGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCC

TTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGT

GAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCAC

TCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT

CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATG

TGTAAAGCATCCCGCTAGCGTTCAATCTGAGCCAGGA

Appendix IVd: Aligned gene sequences details of the PCR amplified product respective to ANT4 gene

AGGGTCCACTTTTCACGCGTTAGCTGCGCTACTAAGGCCTAACGGCCCCAACAGC

TAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC

CACGCTTTCGTGTCTGAGCGTCAGTATTATCCCAGGGGGCTGCCTTCGCCATCGG

TATTCCTCCACATATCTACGCATTTCACTGCTACACGTGGAATTCTACCCCCCTCT

GACATACTCTAGCTCGGCAGTTAAAAATGCAGTTCCAAGGTTGAGCCCTGGGATT

TCACATCTTTCTTTCCGAACCGCCTACACACGCTTTACGCCCAGTAATTCCGATTA

ACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTA

TTCTGCAGATACCGTCAGCAGCATCCCGTATTAGGGGATACCTTTTCTTCTCTGCC

AAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCA

GGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGC

CGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTG

CCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATA

GTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCC

ACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCCGATATATTACTCACC

CGTCCGCCACTCGCCGGCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGC

ATGTGTAAAGCATCCCGCTAGCGTTCAATCTGAGCCAGGAATCAAAACTCTACAC

Appendix IVe: Aligned gene sequences details of the PCR amplified product respective to PYR5 gene

AGCGGGGAAACTTATCACTTTCGCTTAGCCACTCAGGCCGAAAACCGAACAGCT

AGTATCCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTGCGCTACCC

ACGCTTTCGTTCATCAGCGTCAATAAGTACGTAGTAATCTGCCTTCGCAATTGGT

ATTCCATGTAATATCTAAGCATTTCACCGCTACACTACATATTCTAATTACTTCCA

TACTATTCAAGTCTAGCAGTATCAACGGCAATTTTACAGTTAAGCTGTAAGATTT

CACCACTGACTTACTAAACCGCCTACGAACCCTTTAAACCCAATAATTCCGGATA

ACGCTCGGATCCTCCGTATTACCGCGGGTGCTGGGACGGAGTTAGCCGATCCTTA

TTCTTACGGTACCGTCAAATAATTACACGTAATTACATTTCTTCCCGTACAAAAG

CAGTTTACAATCCATAGGACCGTCATCCTGCACGCGGCATGGGTGGTTCAGAGAT

GCCTCCATTGACCAATATTCCTCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGT

CTCAGGACCAGTGTGGGGGATCTCCCTCTCAGGACCCCTAATCATCGTTGCCTTG

GGATGCCGTTACCACACCAACTAGCTAATGATACGCATGCCCATCTTTTACCGAT

AAATCTTTATTATAAATGAGATGCCTCATCTATAAACCATGGAGCATTAATCCGA

ATTTCTCCGGGCTATTCCCCTGTAAAAGGTAGGTTGCATACGCGTTACTCACCCA

TCCGCCGGTCTCAAAAAAGCAAGATCTCTCTACCCCTCGACTTGCATGTGTTAGG

CCTGCCGCTAGCGTTCATCCTGAGCCACGATTCAAACTCTGGAGCAAGCTCTA

Appendix IVf: Aligned gene sequences details of the PCR amplified product respective to ANT6 gene

CGCCGTTGTAGCCTCCCGGTAGCCACGCCTCAAGGGCACAACCTCCAAATCGAC

AACGCCTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTT

TCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCT

CCAGATCTCTACGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACGAGA

CTCTAGCTTGCCAGTTTCAAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACAT

CTGACTTAACAAACCGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATTAACGCT

TGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTG

CGAGTAACGTCAATTGATGAACGTATTAAGTTCACCACCTTCCTCCTCGCTGAAA

GTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTT

GCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGT

CTCAGTTCCAGTGTGGCTGGTCATCCTCCTCAGACCAGCTAGGGATCGTCGCCTA

GGTGAGCCATTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCAA

GAGGCCCGAAGGTCCCCCCTCTYTGGTCTTGCGACGTTATGCGGTATTAGCTACC

GTTTCCAGTAGTTATCCTCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTC

CGCCGCTCGTCACCCAGGGAGCAAGCTCCCCTGTGCTACCGCTTCGACTTGCATG

CGTTAAGCCTGCCGCCAGCGTTCAATCTGAGCCAGGATTCAAACTCT

Appendix IVg: Aligned gene sequences details of the PCR amplified product respective to XYL7 gene

ACCGAATTTAAACGGCGTTAGCTCCGAAAAGCCACTCCTCTAGGGAACAACCTTC

AAATCGACAGCGTTTACAGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCC

CACGCTTTCGCACCTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGG

TATTCCTCCACATCTCTACGCATTTCACCGCTACACATGGAATTCTACCCCCCTCT

ACAAGACTCTAGCTGACCAGTCTTAGATGCCATTCCCAGGTTAAGCCCGGGGATT

TCACATCTAACTTAATCAACCGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATT

AACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTT

CTTCTGTCGGTAACGTCAATCGTTGATGATATTAGCATCAGCGCCTTCCTCCCGAC

TGAAAGTACTTTACAACCCTAGGGCCTTCTTCATACACGCGGCATGGCTGCATCA

GGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGC

CGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCG

CCTAGGTGAGCCATTACCTCACCTACTAGCTAATCCCATATGGGTTCATCCGATA

GCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTGAGAGATTATGCGGTATTAGCT

ACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCCATACATTTACTCAC

CCCGTTCCGCCCGCTCGTCAGCGAGAAGCAAGCTTTCCCCTGTTACCGCTCGACT

TGCATGTGTTACGCCTGCCGCCAGCGTTCAATCTGAGCCAGGATCCAACTCTAGT

Appendix IVh: Aligned gene sequences details of the PCR amplified product respective to XYL8 gene

GGGGAGTGGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACA

CTTAGCATTCAAACGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCT

CCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCAC

TGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCC

TCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGG

CTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCG

GATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGG

CTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCC

CTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCC

GTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCT

GGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATC

GTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCC

ATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAATGTTAT

CCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCA

CGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCCAGCTCTTAATCCATTC

GCTCGACTTGCATGTATTACGCACGCCGCCAGCGTTTCATCCTGAGCCAGGATCA

AAACTCCT

Appendix IVi: Aligned gene sequences details of the PCR amplified product respective to PYR9 gene

Appendix V: Metabolites

The result of the retention factor of the aromatic hydrocarbons degraded by marine bacterial isolates is presented in Appendix Va.

Appendix Va: Retention factor (R_f)of the aromatic hydrocarbons degraded by marine bacterial isolates

Isolate	AHs		
	Xylene	Anthracene	Pyrene
ANT1	0.27 ± 0.08	0.74 ± 0.08	0.34 ± 0.08
XYL2	0.69 ± 0.07	0.86 ± 0.01	0.24 ± 0.02
PYR3	0.76 ± 0.08	0.86 ± 0.01	1.00 ± 0.12
ANT4	0.82 ± 0.08	0.98 ± 0.08	0.83 ± 0.08
PYR5	0.82 ± 0.08	1.00 ± 0.12	0.92 ± 0.01
ANT6	0.71± 0.01	0.41 ± 0.01	0.59 ± 0.01
XYL7	0.84 ± 0.01	0.82 ± 0.08	0.38 ± 0.01
XYL8	0.32 ± 0.01	1.00 ± 0.12	0.79 ± 0.01
PYR9	0.30 ± 0.01	0.88 ± 0.01	0.06 ± 0.03
Control	0.50 ± 0.01	0.63 ± 0.02	0.38 ± 0.01

KEY: Retention factor (R_f) $= \frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$

AHs= Aromatic hydrocarbons

Appendix VI: Statistical Analysis

The results of the statistical analysis of the ANOVA analysis of total aromatic hydrocarbon contents, total heavy metal concentrations, general parameter of sediment samples, general parameter of water samples, relationship between positive control ($K_2Cr_2O_7$), samples and concentrations during 72 hrs inhibition response of *Phaeodactylum tricornutum*, ANOVA analysis of microalgae, germination index, Relationship between positive control ($K_2Cr_2O_7$), samples and concentrations during 24 hrs lethal response of *Artemia franciscana*, ANOVA analysis of *Artemia franciscana*, ANOVA analysis of bacterial assay, relationship between positive control ($HgCl_2$), samples and concentrations during 1.5 hrs toxic response of mutant *E. coli*, T - test analysis of mice body weight, ANOVA analysis of aromatic hydrocarbon organ weights, aromatic hydrocarbon haematological indices, aromatic hydrocarbon biochemical indices, total bacterial count, relationship between concentrations of aromatic hydrocarbons samples and the marine bacterial strains during the concentration effect study, ANOVA analysis of the aromatic hydrocarbon concentration effect, relationship between percentage degradation of hydrocarbons by marine bacterial strains and incubation days during the 24 days degradation assay and ANOVA analysis of the aromatic hydrocarbon degradations are presented in Appendices VIa, VIb, VIc, VId, VIe, VIf, VIg, VIh, VIi, VIj, VIk, VIl, VIm, VIn, VIo, VIp, VIq, VIr, VIs and VIt.

Appendix VIa: ANOVA analysis of the total aromatic hydrocarbon contents

1	Table Analyzed	Total Aromatic hydrocarbon fraction
2		
3	ANOVA summary	
4	F	1.729
5	P value	0.144
6	P value summary	ns
7	Are differences among means statistically significant? (P < 0.05)	No
8	R square	0.1358
9		
10	Brown-Forsythe test	
11	F (DFn, DFd)	1.374 (5, 55)
12	P value	0.248
13	P value summary	2 ns
14	Significantly different standard deviations? (P < 0.05)	No
15		
16	Bartlett's test	
17	Bartlett's statistic (corrected)	110.0
18	P value	< 0.0001
19	P value summary	****
20	Significantly different standard deviations? (P < 0.05)	*
21		Yes
22	ANOVA table	
23	Treatment (between columns)	SS 0.001952, DF 5, MS 0.0003903, F (DFn, DFd) = F (5, 55) = 1.729, P value = P = 0.1434
24	Residual (within columns)	SS 0.01242, DF 55, MS 0.0002258
25	Total	SS 0.01437, DF 60
26		
27	Data summary	
28	Number of treatments (columns)	6
29	Number of values (total)	61

Appendix VIb: ANOVA analysis of the total heavy metal concentrations

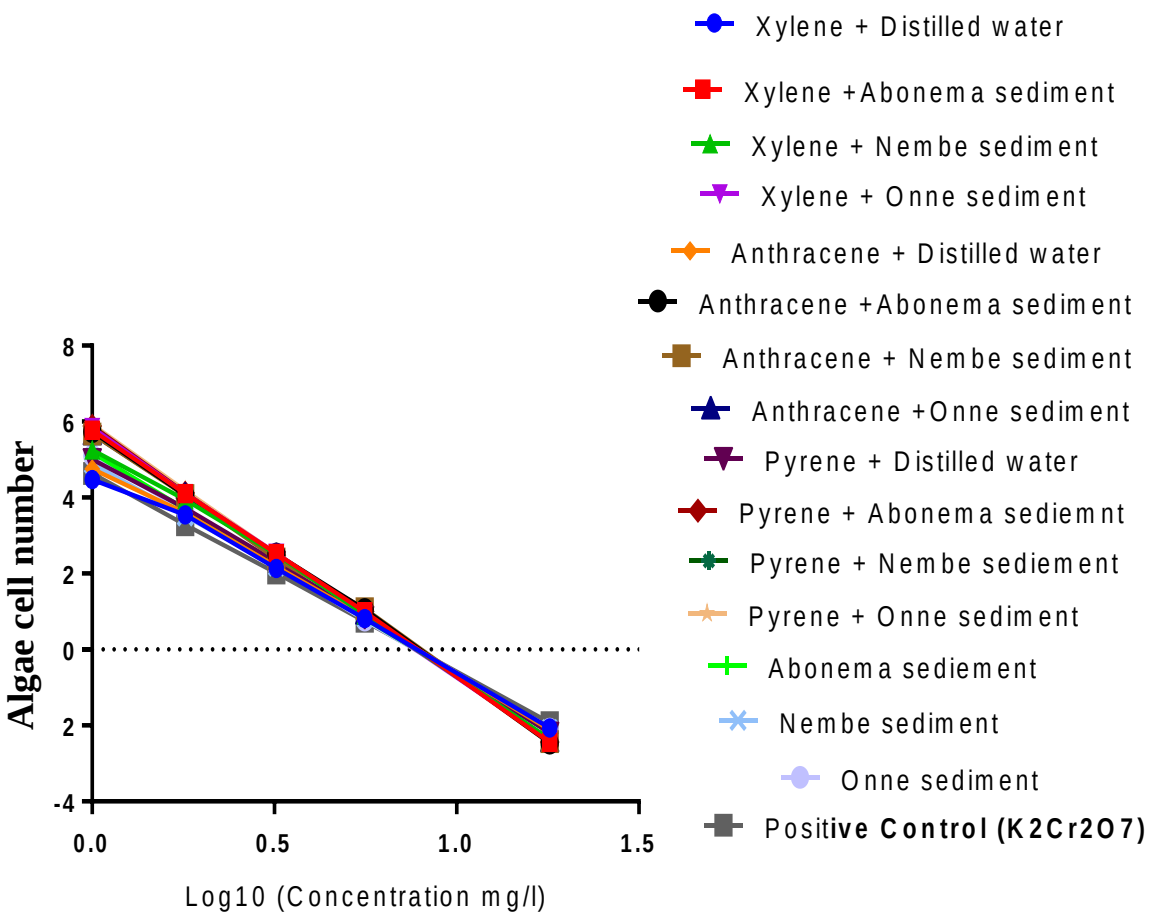
	Table Analyzed	Total heavy metal concentration			
2	ANOVA summary				
4	F	0.6389			
5	P value	0.679			
6	P value summary	ns			
7	Are differences among means statistically significant? (P < 0.05)	No			
8	R square	0.05055			
9					
10	Brown-Forsythe test				
11	F (DFn, DFd)	0.6299 (5, 60)			
12	P value	0.676			
13	P value summary	ns			
14	Significantly different standard deviations? (P < 0.05)	No			
15					
16	Bartlett's test				
17	Bartlett's statistic (corrected)	164.3			
18	P value	< 0.0001			
19	P value summary	****			
20	Significantly different standard deviations? (P < 0.05)	Yes			
21					
22	ANOVA table	SS	D	MS	F(DFn, DFd)	P value
23	Treatment (between columns)	1359	5	271.9	F (5, 60) = 0.6389	P = 0.6709
24	Residual (within columns)	25533	60	425.5		
25	Total	26892	65			
26						
27	Data summary					
28	Number of treatments (columns)	6				
29	Number of values (total)	66				

Appendix VIc: ANOVA analysis of general parameter of sediment samples

1	Table Analyzed	General parameters of sediment samples				
2						
3	ANOVA summary					
4	F	1.064				
5	P value	0.355				
6	P value summary	0 ns				
7	Are differences among means statistically significant? (P < 0.05)	No				
8	R square	0. 05172				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	1.058 (2,				
12	P value	0.3570				
13	P value summary	ns				
14	Significantly different	No				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)	126.5				
18	P value	< 0.0001				
19	P value summary	****				
20	Significantly different standard deviations? (P < 0.05)	Yes				
21						
22	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
23	Treatment (between	146595	2	73297		
24	Residual (within columns)	2.688e+006	39	68914	F (2, 39) = 1.064	P = 0.3550
25	Total	2.834e+006	41			
26						
27	Data summary					
28	Number of treatments (columns)	3				
29	Number of values (total)	42				

Appendix VId: ANOVA analysis of general parameter of water samples

1	Table Analyzed	General parameters of water samples				
3	ANOVA summary					
4	F	0.251				
5	P value	0.7796				
6	P value summary	ns				
7	Are differences among means statistically significant? (P < 0.05)	No				
8	R square	0.0165				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	0.240 (2, 30)				
12	P value	0.7880				
13	P value summary	ns				
14	Significantly different standard deviations? (P < 0.05)	No				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)	6.67				
18	P value	0.0356				
19	P value summary	*				
20	Significantly different standard deviations? (P < 0.05)	Yes				
21						
22	ANOVA table	SS	DF	MS	F (DFn, Dfd)	P value
23	Treatment (between columns)	1210	2	605	F (2, 30) = 0.251	P = 0.7796
24	Residual (within columns)	72288	30	2410		
25	Total	73498	32			
26						
27	Data summary					
28	Number of treatments (columns)	3				
29	Number of values (total)	33				

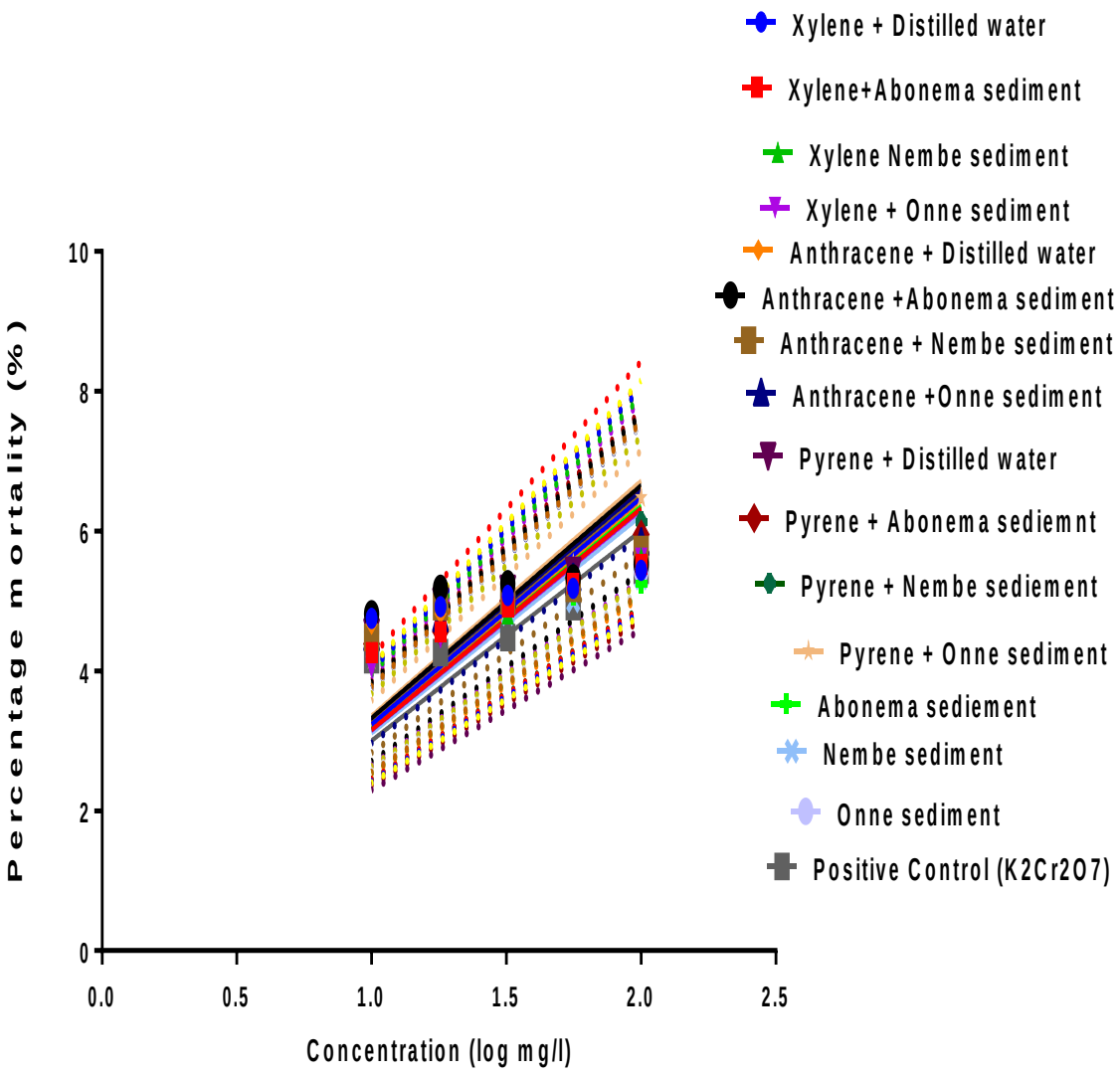


Appendix VIe: Relationship between positive control ($K_2Cr_2O_7$), samples and concentrations during 72 h inhibition response of *Phaeodactylum tricornutum*

Appendix VIf: ANOVA analysis of microalga

1	Table Analyzed	Marine microalgae				
2						
3	ANOVA summary					
4	F	0.732				
5	P value	0.7453				
6	P value summary	ns				
7	Are differences among means statistically significant? (P < 0.05)	No				
8	R square	0.119				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	0.268 (15, 81)				
12	P value	0.9969				
13	P value summary	ns				
14	Significantly different standard deviations? (P < 0.05)	No				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)	7.91				
18	P value	0.9272				
19	P value summary	ns				
20	Significantly different standard deviations? (P < 0.05)	No				
21						
22	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
23	Treatment (between columns)	20.5	15	1.37	F (15, 81) = 0.732	P = 0.7453
24	Residual (within columns)	151	81	1.87		
25	Total	172	96			
26						
27	Data summary					
28	Number of treatments (columns)	16				
29	Number of values (total)	97				

1	Table Analyzed	Germination index			
3	ANOVA summary				
4	F	54.27			
5	P value	< 0.0001			
6	P value summary	****			
7	Are differences among means Statistically significant? (P < 0.05)	Yes			
8	R square	0.9622			
9					
10	Brown-Forsythe test				
11	F (DFn, DFd)	0.9944 (15, 32)			
12	P value	0.4836			
13	P value summary	ns			
14	Significantly different standard deviations? (P < 0.05)	No			
15	ANOVA table	SS	D	MS	
16	Treatment (between columns)	2068	1	137.9	F (DFn, DFd) P value
17	Residual (within columns)	81.29	3	2.540	F (15, 32) = P<0.0001
18	Total	2149	4		54.27
19					
20	Model comparison	SS	D	Probabil	
21	Null H. All population means identical	2149	F 4	it is correct 0.00%	
22	Alternative H: Distinct population means	81.29	3 2	100.00%	
23	Ratio of probabilities			0.0	
24	Difference in AICc			107.1	
25	Data summary				
26	Number of treatments	16			
27	Number of values (total)	48			



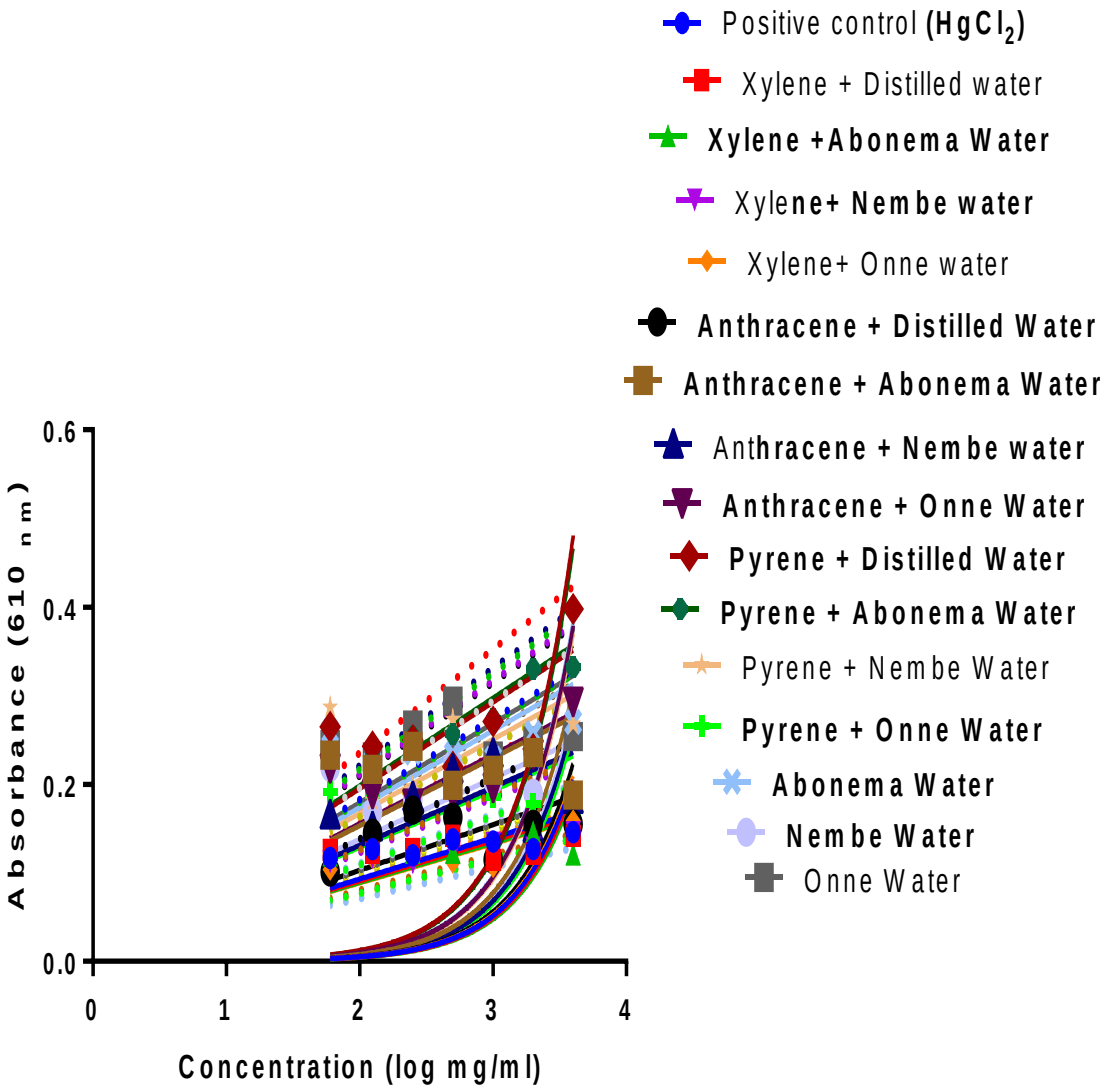
Appendix VIh: Relationship between positive control ($K_2Cr_2O_7$), samples and concentrations during 24 h lethal response of *Artemia franciscana*

Appendix VIi: ANOVA analysis of *Artemia franciscana*

1	Table Analyzed	Percent mortality				
2						
3	ANOVA summary					
4	F	0.829				
5	P value	0.6440				
6	P value summary	ns				
7	Are differences among means statistically significant? (P < 0.05)	No				
8	R square	0.109				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	0.741 (15, 102)				
12	P value	0.7375				
13	P value summary	ns				
14	Significantly different standard deviations? (P < 0.05)	No				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)	250				
18	P value	< 0.0001				
19	P value summary	****				
20	Significantly different standard deviations? (P < 0.05)	Yes				
21						
22	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
23	Treatment (between columns)	1265	15	84.4	F (15, 102) = 0.829	P = 0.6440
24	Residual (within columns)	10384	102	102		
25	Total	11649	117			
26						
27	Data summary					
28	Number of treatments (columns)	16				
29	Number of values (total)	118				

Appendix VIj: ANOVA analysis of bacterial assay

1	Table Analyzed	Bacterial assay				
2						
3	ANOVA summary					
4	F	26.9				
5	P value	< 0.0001				
6	P value summary	****				
7	Are differences among means statistically significant? (P < 0.05)	Yes				
8	R square	0.808				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	1.55 (15, 96)				
12	P value	0.1016				
13	P value summary	ns				
14	Significantly different standard deviations? (P < 0.05)	No				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)	57.3				
18	P value	< 0.0001				
19	P value summary	****				
20	Significantly different standard deviations? (P < 0.05)	Yes				
21						
22	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
23	Treatment (between columns)	0.323	15	0.0216	F (15, 96) = 26.9	P < 0.0001
24	Residual (within columns)	0.0770	96	0.000802		
25	Total	0.400	111			
26						
27	Data summary					
28	Number of treatments (columns)	16				
29	Number of values (total)	112				



Appendix VIk: Relationship between positive control ($HgCl_2$), samples and concentrations during 1.5 h toxic response of mutant *E. coli*

Appendix VIl: T-test analysis of mice body weight

1	Table Analyzed	Aromatic hydrocarbons
2		
3	Column C	After (g)
4	vs.	vs.
5	Column B	Before (g)
6		
7	Paired t test	0.221
8	P value	6 ns
9	P value summary	No
10	Significantly different? (P < 0.05)	Two-tailed
11	One- or two-tailed P value?	t=1.538 df = 3
12	t, df	4
13	Number of pairs	
14		
15	How big is the difference?	
16	Mean of differences	-1.298
17	SD of differences	1.687
18	SEM of differences	0.8434
19	95% confidence interval	-3.982 to 1.387
20	R square	0.4410
21		
22	How effective was the pairing?	0.8474
23	Correlation coefficient (r)	0.076
24	P value (one tailed)	3 ns
25	P value summary	
26	Significant correlation? (P > 0.05)	Yes

Appendix VIm: ANOVA analysis of aromatic hydrocarbon organ weights

1	Table Analyzed	Aromatic hydrocarbons				
2						
3	Repeated measures ANOVA					
4	Assume sphericity?	No				
5	F	0.6197				
6	P value	0.4887				
7	P value summary	ns				
8	Statistically significant (P < 0.05)?	No				
9	Geisser-Greenhouse's epsilon	0.5002				
10	R square	0.1712				
11						
12	Was the matching effective?					
13	F	0.8				
14	P value	0.5				
15	P value summary	ns				
16	Is there significant matching	? No				
17	(P < 0.05)	0.2				
18						
19	ANOVA table	SS	DF	M	F (DFn,	P value
20	Treatment (between columns)	82 0.2	2	41 0.1	F (1.000, 3.001) = 0.6197	P = 0.4887
21	Individual (between rows)	16 86	3	56 1.9	F (3, 6) = 0.8492	P = 0.5156
22	Residual (random)	39	6	66		
23	Total	64	11			
24						
25	Data summary					
26	Number of treatments	3				
27	Number of subjects (rows)	4				

Appendix VIn: ANOVA analysis of aromatic hydrocarbon haematological indices

Data analyzed: Hydrocarbon haematology

Source of Variation	Degrees of	Sum of	Mean
haematological	3.0	8513	2838
doses	9.0	379047	42116
Residual (error)	27.0	71246	2639
Total	39.0	458805	

Because you have not provided replicate values, it is necessary to ASSUME that there is no interaction. In other words, this analysis assumes that haematological parameters has the same effect (if any) at all levels of doses.

Does haematological parameters affect the result?

Haematological parameters accounts for 1.86% of the total variance. F = 1.08.

DFn=3 DFd=27

The P value = 0.3761

If haematological parameters has no effect overall, there is a 38% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered not significant.

Does doses affect the result?

Doses accounts for 82.62% of the total variance. F = 15.96. DFn=9 DFd=27

The P value is < 0.0001

If doses has no effect overall, there is a less than 0.01% chance of randomly observing a big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Appendix VIo: ANOVA analysis of aromatic hydrocarbon biochemical indices

Data analyzed: **Hydrocarbon biochemical**

Source of Variation	Degrees of Freedom	Sum of Squares	Mean square
biochemical	3.0	6597	2199
Doses	12.0	252965	21080
Residual (error)	36.0	24315	675.4
Total	51.0	283877	

Because you have not provided replicate values, it is necessary to ASSUME that there is no interaction. In other words, this analysis assumes that biochemical parameters has the same effect (if any) at all levels of Doses.

Does biochemical parameters affect the result?

Biochemical parameters accounts for 2.32% of the total variance. F = 3.26. DFn=3 DFd=36

The P value = 0.0327

If biochemical parameters has no effect overall, there is a 3.3% chance of randomly observing an effect this big (or bigger) in an experiment of this size.

The effect is considered significant.

Does Doses affect the result?

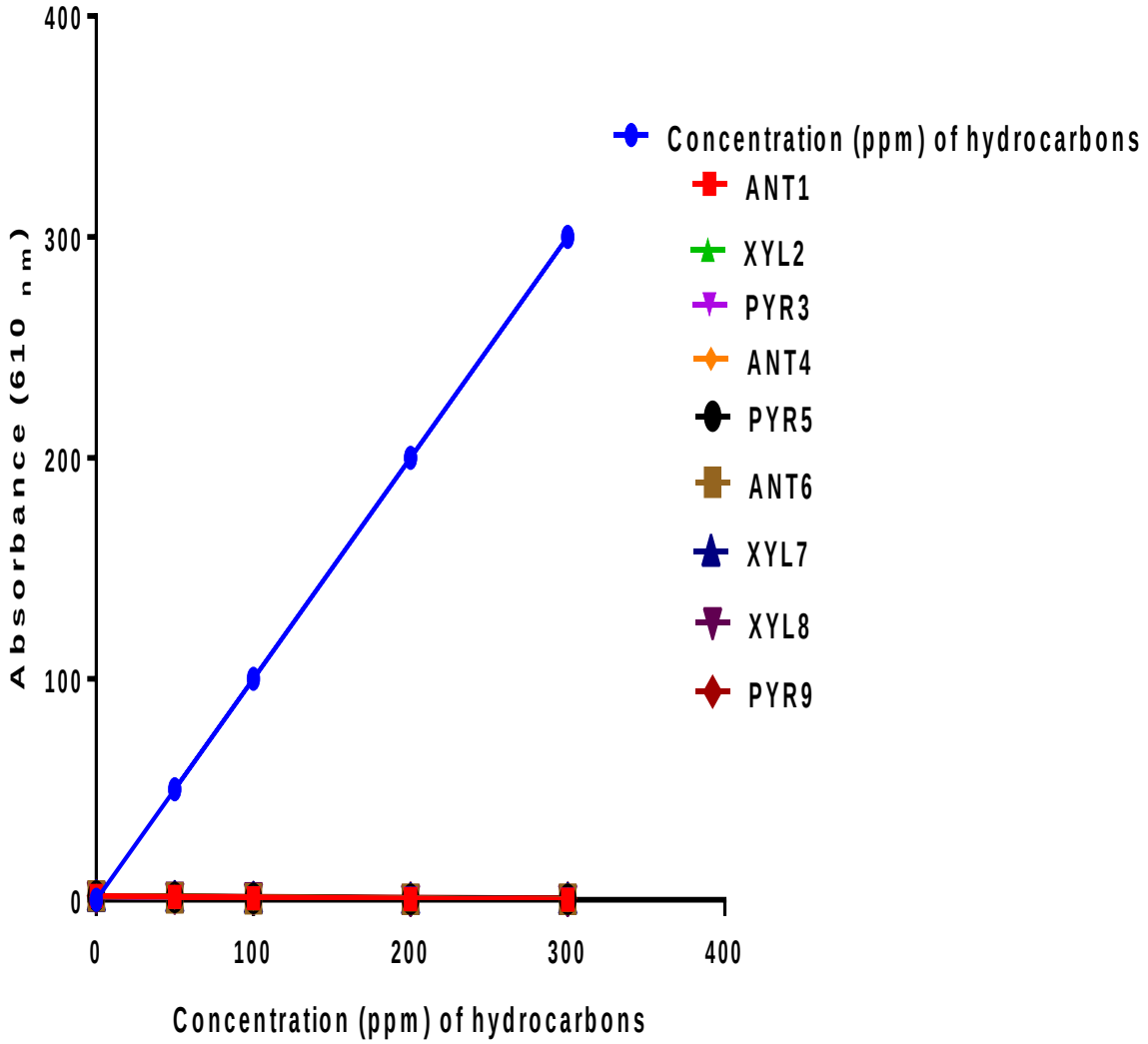
Doses accounts for 89.11% of the total variance. F = 31.21. DFn=12 DFd=36

The P value is < 0.0001

If Doses has no effect overall, there is a less than 0.01% chance of randomly observing an this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Appendix VIp: ANOVA analysis of total bacterial count

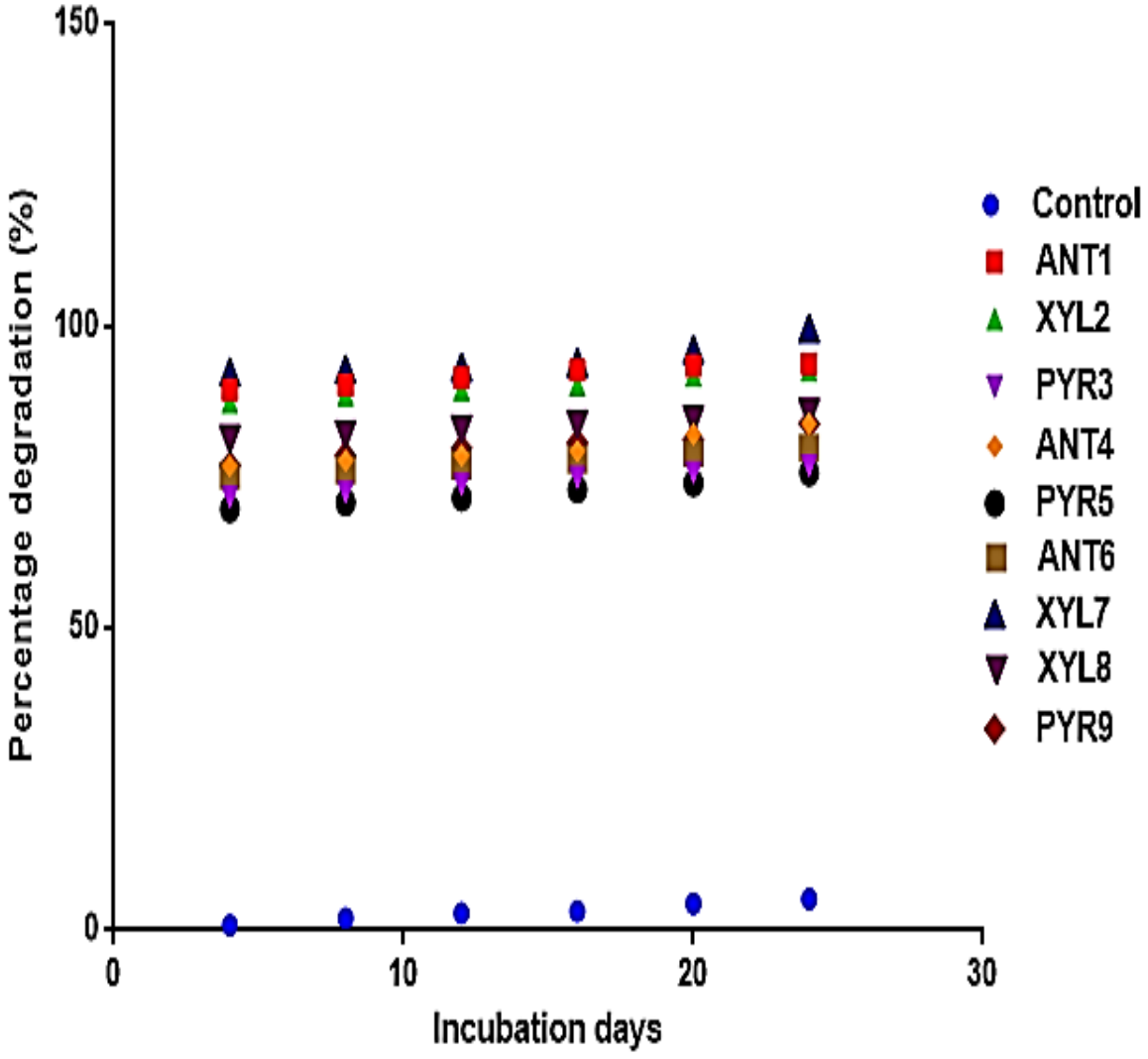
1	Table Analyzed	TBC				
2						
3	ANOVA summary					
4	F	15.4				
5	P value	< 0.0001				
6	P value summary	****				
7	Are differences among means statistically significant? (P < 0.05)	Yes				
8	R square	0.773				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	0.785 (17, 77)				
12	P value	0.7045				
13	P value summary	ns				
14	Significantly different standard deviations? (P < 0.05)	No				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)					
18	P value					
19	P value summary					
20	Significantly different standard deviations? (P < 0.05)					
21						
22	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
23	Treatment (between columns)	2826	17	166	F (17, 77) = 15.4	P < 0.0001
24	Residual (within columns)	832	77	10.8		
25	Total	3657	94			
26						
27	Model comparison	SS	DF	Probal		
28	Null H. All population means identical	3657	94	0.00%		
29	Alternative H: Distinct population means	832	77	100.0[		
30	Ratio of probabilities			0.0		
31	Difference in AICc			96.7		
32						
33	Data summary					
34	Number of treatments (columns)	18				
35	Number of values (total)	95				



Appendix VIq: Relationship between concentrations of aromatic hydrocarbons samples and the marine bacterial strains during the concentration effect study

Appendix VIr: ANOVA analysis of the aromatic hydrocarbon concentration effect

1	Table Analyzed	AH Conc. effect				
2						
3	ANOVA summary					
4	F	5.75				
5	P value	< 0.0001				
6	P value summary	****				
7	Are differences among means statistically significant? (P < 0.05)	Yes				
8	R square	0.564				
9						
10	Brown-Forsythe test					
11	F (DFn, DFd)	7.29 (9, 40)				
12	P value	< 0.0001				
13	P value summary	****				
14	Significantly different standard deviations? (P < 0.05)	Yes				
15						
16	Bartlett's test					
17	Bartlett's statistic (corrected)	266				
18	P value	< 0.0001				
19	P value summary	****				
20	Significantly different standard deviations? (P < 0.05)	Yes				
21						
22	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
23	Treatment (between columns)	75021	9	8336	F (9, 40) = 5.75	P < 0.0001
24	Residual (within columns)	58013	40	1450		
25	Total	133034	49			
26						
27	Data summary					
28	Number of treatments (columns)	10				
29	Number of values (total)	50				



Appendix VIs: Relationship between percentage degradation of hydrocarbons by marine bacterial strains and incubation days during the 24 days' degradation asssay

Appendix VIt: ANOVA analysis of the aromatic hydrocarbon degradations

Data analyzed: Aromatic hydrocarbon degradations

Source of Variation	Degrees of Freedom	Sum of	Mean
Incubation days	9.0	9487	1054
Degraders	5.0	985	197
Residual (error)	4 5.0	70.5	1.57
Total	5 9.0	10542	

Because you have provided replicate values, it is necessary to ASSUME that there is interaction. In other words, this analysis assumes that the Degraders do not have the same effect (if any) at all levels of Incubation days.

Does Incubation days affect the result?

Incubation days accounts for 89.99% of the total variance. F = 672.45. DFn=9 DFd=45

The P value is < 0.0001

If Incubation days has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Does Degraders affect the result?

Degraders accounts for 9.34% of the total variance. F = 125.62. DFn=5 DFd=45

The P value is < 0.0001

If Degraders has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.